DESCRIPTION

Title of Invention: ENZYME AGENT FOR PROTEIN DEAMIDATION AND PROTEIN CROSS-LINKING AND INTENDED USE THEREOF

Technical Field

[0001]

The present invention relates to an enzyme agent (an enzyme agent for protein deamidation and protein cross-linking), which contains an enzyme having a protein deamidation activity and a transglutaminase activity as an active ingredient, and intended use thereof.

Background Art

[0002]

Protein deamidase (For example, protein glutaminase (EC number: 3.5.1.44)) is an enzyme that acts on a protein, which is a macromolecule and catalyzes a deamidation reaction. Protein deamidase deamidates glutamine residues in a protein and allows negatively charged carboxyl groups to generate, thereby bringing about various property changes to the protein. For example, an increase in hydration strength and an increase in electrostatic repulsion, which are caused by a decrease in the isoelectric point of the protein, reduce protein-protein interactions (i.e., reduce association), thus increasing the solubility and water dispersibility of the protein. Moreover, the exposure of internal hydrophobic regions due to changes in the higher-order structure of protein imparts surface activity to the protein, thereby improving the emulsifying power, emulsification stability, foaming ability, and foam stability of the protein. Since protein deamidases can significantly alter the properties of proteins in this way, they have dramatically increased the intended uses of proteins. Accordingly, protein deamidase is highly useful and has attracted considerable interest in this technological field.

[0003]

On the other hand, transglutaminase (EC number: 2.3.2.13) (also referred to as TG) is an

enzyme that catalyzes the acyl transfer reaction of the γ-carboxylamide groups of glutamine residues in peptide chains. When the ε-amino group of lysine residues in a protein acts as an acyl acceptor, transglutaminase forms an ε-(γ-Gln)-Lys cross-linking bond within or between protein molecules. Since the action of transglutaminase can be utilized to modify proteins or peptides, the transglutaminase is used for the binding of meat, and for production of sausages, *tofu*, bread, and noodles. Moreover, the utilization of transglutaminase is being explored not only in the field of food products, but also in the fields of textile, medical care, and cosmetic products, and other fields.
[0004]

Protein deamidase and transglutaminase are common in that they act on glutamine residues, but they are different enzymes having different EC numbers. Transglutaminase catalyzes the deamidation reaction of glutamine residues in the absence of acyl receptors, but hardly catalyzes the deamidation reaction in the presence of acyl receptors such as proteins. Thus, if both the protein deamidation reaction and the transglutaminase reaction have been desired to be catalyzed, it has been necessary to use separately prepared enzymes in combination. For example, Patent Document 1 discloses a method for modifying proteins by allowing protein glutaminase and transglutaminase to act on the proteins, and this patent document describes that, in the production of yogurt using milk, the production of tofu using soy milk, and the production of noodles using wheat, the smoothness of the produced foods have been improved by treatment with protein glutaminase and transglutaminase.

Prior Art Documents

Patent Documents

[0005]

Patent Document 1: International Publication WO2009/113628

Summary of Invention

Objects to be Solved by the Invention

[0006]

As mentioned above, it can be expected that protein-modifying effects are obtained by using a protein glutaminase and a transglutaminase in combination, but the enzymes that can be

utilized has been limited. In addition, according to conventional technology, it has been necessary to use two types of enzymes in combination in order to proceed with both the protein deamidation reaction and the transglutaminase reaction, which has been problematic in that the production process has become complicated. Thus, it is an object of the present invention to provide a novel enzyme agent containing, as an active ingredient, an enzyme having both a protein deamidation activity and a transglutaminase activity.

Means for Solving the Objects

[0007]

The present inventor has newly found that a polypeptide derived from *Longimycelium tulufanense* has both a protein deamidation activity and a transglutaminase activity. The present invention has been completed based on the above-described findings.

[0008]

According to the present invention, the following inventions are provided.

[1] An enzyme agent for protein deamidation and protein cross-linking, the enzyme agent containing, as an active ingredient, an enzyme that consists of an amino acid sequence having a sequence identity of 90% or more to the amino acid sequence as set forth in SEQ ID NO: 1 and has a protein deamidation activity and a transglutaminase activity.

[2] The enzyme agent for protein deamidation and protein cross-linking according to [1], in which the enzyme is derived from Longimycelium sp.

[3] A method for deamidating and cross-linking a protein or a peptide, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [1] or [2] to act on the protein or the peptide.

[4] A method for deamidating and cross-linking protein-containing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [1] or [2] to act on the protein-containing food product and beverage.

[5] A method for producing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to

[1] or [2] to act on protein-containing food product and beverage.

[6] Food product and beverage, treated with the enzyme agent for protein deamidation and protein cross-linking according to [1] or [2].

[7] A method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 90% or more to the amino acid sequence as set forth in SEQ ID NO: 1, including

culturing bacteria of Longimycelium sp.

[8] An enzyme agent for protein deamidation and protein cross-linking, the enzyme agent containing, as an active ingredient, an enzyme that consists of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 6 and has a protein deamidation activity and a transglutaminase activity.

[9] The enzyme agent for protein deamidation and protein cross-linking according to [8], in which the enzyme is derived from Crossiella sp.

[10] A method for deamidating and cross-linking a protein or a peptide, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [8] or [9] to act on the protein or the peptide.

[11] A method for deamidating and cross-linking protein-containing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [8] or [9] to act on the protein-containing food product and beverage.

[12] A method for producing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [8] or [9] to act on protein-containing food product and beverage.

[13] Food product and beverage, treated with the enzyme agent for protein deamidation and protein cross-linking according to [8] or [9].

[14] A method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 6, including

culturing bacteria of Crossiella sp.

[15] An enzyme agent for protein deamidation and protein cross-linking, the enzyme agent containing, as an active ingredient, an enzyme that consists of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 10 and has a protein deamidation activity and a transglutaminase activity.

[16] The enzyme agent for protein deamidation and protein cross-linking according to [15], in which the enzyme is derived from Krasilnikovia sp.

[17] A method for deamidating and cross-linking a protein or a peptide, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [15] or [16] to act on the protein or the peptide.

[18] A method for deamidating and cross-linking protein-containing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [15] or [16] to act on the protein-containing food product and beverage.

[19] A method for producing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to [15] or [16] to act on protein-containing food product and beverage.

[20] Food product and beverage, treated with the enzyme agent for protein deamidation and protein cross-linking according to [15] or [16].

[21] A method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 10, including

culturing bacteria of Krasilnikovia sp.

[0009]

< A > Use of an enzyme agent for protein deamidation and protein cross-linking, the enzyme agent containing as an active ingredient, an enzyme that consists of an amino acid sequence having a sequence identity of 90% or more to the amino acid sequence as set forth in SEQ ID NO: 1 and has a protein deamidation activity and a transglutaminase activity.

Advantageous Effects of Invention

[0010]

The enzyme agent for protein deamidation and protein cross-linking according to the present invention, containing an enzyme having a protein deamidation activity and a transglutaminase activity, is suitable to be used in the fields of food products and medical applications.

Brief Description of Drawings

[0011]

[Figure 1] Figure 1 shows the results of evaluation of the stickiness of the enzyme-treated casein protein in the example and comparative example.

Embodiments of Carrying out the Invention

[0012]

1. Enzyme agent for protein deamidation and protein cross-linking

The enzyme agent for protein deamidation and protein cross-linking of a first embodiment of the present invention contains, as an active ingredient, an enzyme consisting of an amino acid sequence consisting of the amino acid sequence as set forth in SEQ ID NO: 1, or an amino acid sequence equivalent thereto, and having a protein deamidation activity and a transglutaminase activity (hereinafter also referred to as a "protein deamidation and protein cross-linking enzyme" or "the present enzyme").

[0013]

The enzyme agent for protein deamidation and protein cross-linking of a second

embodiment of the present invention contains, as an active ingredient, an enzyme consisting of an amino acid sequence consisting of the amino acid sequence as set forth in SEQ ID NO: 6, or an amino acid sequence equivalent thereto, and having a protein deamidation activity and a transglutaminase activity (hereinafter also referred to as a "protein deamidation and protein cross-linking enzyme" or "the present enzyme").

[0014]

The enzyme agent for protein deamidation and protein cross-linking of a third embodiment of the present invention contains, as an active ingredient, an enzyme consisting of an amino acid sequence consisting of the amino acid sequence as set forth in SEQ ID NO: 10, or an amino acid sequence equivalent thereto, and having a protein deamidation activity and a transglutaminase activity (hereinafter also referred to as a "protein deamidation and protein cross-linking enzyme" or "the present enzyme").

[0015]

Besides, the present invention may relate to an agent for protein deamidation and protein cross-linking containing, as an active ingredient, an enzyme consisting of an amino acid sequence consisting of the amino acid sequence as set forth in SEQ ID NO: 14, or an amino acid sequence equivalent thereto, and having a protein deamidation activity and a transglutaminase activity (hereinafter also referred to as a "protein deamidation and protein cross-linking enzyme" or "the present enzyme") (a fourth embodiment).

[0016]

The protein deamidation and protein cross-linking enzyme of the present invention is an enzyme exhibiting an action to decompose or crosslink the amide group-containing side chains of proteins. Specifically, the protein deamidation and protein cross-linking enzyme of the present invention has the activity of deamidating glutamine residues in proteins and converting them to glutamic acid (protein glutaminase activity), and catalyzes the acyl transfer reaction of the γ-carboxylamide group of glutamine residues in proteins. Thereby, modification of proteins can be effectively carried out.

[0017]

The term "equivalent amino acid sequence" means an amino acid sequence that is partially

different from the reference amino acid sequence (i.e. the amino acid sequence as set forth in SEQ ID NO: 1, 6, 10, or 14) but such difference does not substantially influence the function of the protein (which is herein a protein deamidation activity and a transglutaminase activity). Accordingly, an enzyme having such an equivalent amino acid sequence catalyzes an enzyme reaction by an enzyme having a protein deamidation activity and a transglutaminase activity. The degree of the activity is not particularly limited, as long as the function of the enzyme having a protein deamidation activity and a transglutaminase activity can be exhibited. However, the activity of the enzyme having such an equivalent amino acid sequence is preferably equivalent to or higher than the activity of an enzyme consisting of the reference amino acid sequence (i.e. an enzyme having the amino acid sequence as set forth in SEQ ID NO: 1, 6, 10, or 14).

[0018]

In the first embodiment, the protein deamidation and protein cross-linking enzyme is preferably derived from Longimycelium sp., and is more preferably derived from *Longimycelium tulufanense*. The amino acid sequence as set forth in SEQ ID NO: 1 is the amino acid sequence (mature form) of a protein deamidation and protein cross-linking enzyme derived from *Longimycelium tulufanense*. It is to be noted that the full-length amino acid sequence of the protein deamidase and protein cross-linking enzyme derived from *Longimycelium tulufanense* is shown in SEQ ID NO: 2.

[0019]

In the second embodiment, the protein deamidation and protein cross-linking enzyme is preferably derived from Crossiella sp., and is more preferably derived from *Crossiella cryophila*. The amino acid sequence as set forth in SEQ ID NO: 6 is the amino acid sequence (mature form) of a protein deamidase and protein cross-linking enzyme derived from *Crossiella cryophila*. It is to be noted that the full-length amino acid sequence of the protein deamidase and protein cross-linking enzyme derived from *Crossiella cryophila* is shown in SEQ ID NO: 7.

[0020]

In the third embodiment, the protein deamidation and protein cross-linking enzyme is preferably derived from Krasilnikovia sp., and is more preferably derived from *Krasilnikovia cinnamomea*. The amino acid sequence as set forth in SEQ ID NO: 10 is the amino acid sequence

(mature form) of a protein deamidase and protein cross-linking enzyme derived from *Krasilnikovia cinnamomea*. It is to be noted that the full-length amino acid sequence of the protein deamidase and protein cross-linking enzyme derived from *Krasilnikovia cinnamomea* is shown in SEQ ID NO: 11.

[0021]

In the fourth embodiment, the protein deamidation and protein cross-linking enzyme is preferably derived from Longispora sp., and is more preferably derived from *Longispora fulva*. The amino acid sequence as set forth in SEQ ID NO: 14 is the amino acid sequence (mature form) of a protein deamidase and protein cross-linking enzyme derived from *Longispora fulva*. It is to be noted that the full-length amino acid sequence of the protein deamidase and protein cross-linking enzyme derived from *Longispora fulva* is shown in SEQ ID NO: 15.

[0022]

A "partial difference in an amino acid sequence" is produced, for example, as a result of a deletion or a substitution of one or more amino acids in the amino acids constituting the amino acid sequence, or an addition or an insertion of one or more amino acids into the amino acid sequence, or any given combination thereof. Such a partial difference in the amino acid sequence is acceptable, as long as the protein deamidation activity and transglutaminase activity are retained (in which the activity may be slightly fluctuated). As far as this condition is satisfied, the position of the amino acid sequence, in which the difference is found, is not particularly limited. In addition, such difference may be produced in multiple sites (places) on the amino acid sequence.

[0023]

The number of amino acids providing such a partial difference in the amino acid sequence is a number corresponding to, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10%, a number preferably corresponding to less than about 8%, a number more preferably corresponding to about 6%, a number even more preferably corresponding to less than about 4%, a number further preferably corresponding to less than about 2%, and a number most preferably corresponding to less than about 1%. Accordingly, the equivalent protein has an identity of, for example, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more,

about 81% or more, about 82% or more, about 83% or more, about 84% or more, about 85% or more, about 86% or more, about 87% or more, about 88% or more, about 89% or more, about 90% or more, about 91% or more, about 92% or more, preferably about 93% or more, more preferably about 94% or more, even more preferably about 95% or more, further preferably about 96% or more, even further preferably about 97% or more, still further preferably about 98% or more, and most preferably about 99% or more, with respect to the reference amino acid sequence.

[0024]

A typical example of a "partial difference in the amino acid sequence" is that a mutation (a change) is produced in the amino acid sequence, as a result of a deletion or a substitution of 1 to 40 (preferably 1 to 30, more preferably 1 to 10, even more preferably 1 to 7, further preferably 1 to 5, and still further preferably 1 to 3) amino acids in the amino acids constituting the amino acid sequence, or an addition or an insertion of 1 to 40 (preferably 1 to 30, more preferably 1 to 10, even more preferably 1 to 7, further preferably 1 to 5, and still further preferably 1 to 3) amino acids into an amino acid sequence, or any given combination thereof.

[0025]

Preferably, an equivalent amino acid sequence is obtained by the occurrence of conservative amino acid substitution in amino acid residues that are not essential for protein deamidation activity and transglutaminase activity. The term "conservative amino acid substitution" is used herein to mean that a certain amino acid residue is substituted with an amino acid residue having a side chain with similar properties. Amino acid residues are classified into several families, depending on the side chain thereof; namely, basic side chains (for example, lysine, arginine, and histidine), acidic side chains (for example, aspartic acid and glutamic acid), uncharged polar side chains (for example, glycine, asparagine, glutamine, serine, threonine, tyrosine, and cysteine), non-polar side chains (for example, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan), β-branched side chains (for example, threonine, valine, and isoleucine), aromatic side chains (for example, tyrosine, phenylalanine, tryptophan, and histidine), etc. The conservative amino acid substitution is preferably a substitution occurring between amino acid residues in an identical family.

[0026]

The identity (%) of two amino acid sequences can be determined, for example, by the following procedures. First, two sequences are aligned such that an optimal comparison can be made (for example, it may also be possible that a gap is introduced into a first sequence, so that the alignment with a second sequence may be optimized). When a molecule (amino acid residue) in a specific position of the first sequence is identical to a molecule in a corresponding position in the second sequence, it can be said that the molecules at the positions are identical to each other. The identity of the two sequences is a function of the number of identical positions common in the two sequences (i.e. identity (%) = the number of identical positions / total number of positions x 100), and preferably, the number and size of gaps used for optimization of the alignment are also taken into consideration.

[0027]

Comparison of two sequences and determination of identity can be realized using mathematical algorithms. A specific example of the mathematical algorithm that can be utilized in comparison of sequences may be an algorithm that is described in Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87: 2264-68 and is modified in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-77, but the example of the mathematical algorithm is not limited thereto. The identity of amino acid sequences can be obtained, for example, by blastp (protein-protein BLAST) of National Center for Biotechnology Information (NCBI). As parameters, default parameters may be used. For example, using BLOSUM62 matrix, Gap Costs may be set to be Existence: 11 and Extension: 1.

[0028]

The protein deamidation and protein cross-linking enzyme that is an active ingredient of the enzyme agent of the present invention may be a part of a larger protein (for example, a fusion protein). Examples of a sequence to be added to such a fusion protein may include sequences that are useful for purification, such as multiple histidine residues, and additional sequences that ensure stability during recombinant production.

[0029]

The protein deamidation and protein cross-linking enzyme in the first embodiment can be obtained by culturing microorganisms that produce the protein deamidation and protein cross-linking

enzyme (i.e., a strain that produces the present enzyme), for example, the bacteria of Longimycelium sp., such as *Longimycelium tulufanense*. That is to say, the present invention may relate to a method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 90% or more to the amino acid sequence as set forth in SEQ ID NO: 1, in which the method includes a step of culturing bacteria of Longimycelium sp. The strain that produces the present enzyme may be either a wild-type strain or a mutant strain (for example, a mutant strain is obtained by UV irradiation). A specific example of the strain that produces the present enzyme may be *Longimycelium tulufanense* (NBRC107726). *Longimycelium tulufanense* (NBRC107726) is a bacterial strain stored at the NBRC (National Institute of Technology and Evaluation, Biological Resource Center), and it can be obtained by following the prescribed procedures.

[0030]

The protein deamidation and protein cross-linking enzyme in the second embodiment can be obtained by culturing microorganisms that produce the protein deamidation and protein cross-linking enzyme (i.e., a strain that produces the present enzyme), for example, the bacteria of Crossiella sp., such as *Crossiella cryophila*. That is to say, the present invention may relate to a method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 6, in which the method includes a step of culturing bacteria of Crossiella sp. The strain that produces the present enzyme may be either a wild-type strain or a mutant strain (for example, a mutant strain is obtained by UV irradiation).

[0031]

The protein deamidation and protein cross-linking enzyme in the third embodiment can be obtained by culturing microorganisms that produce the protein deamidation and protein cross-linking enzyme (i.e., a strain that produces the present enzyme), for example, the bacteria of Krasilnikovia sp., such as *Krasilnikovia cinnamomea*. That is to say, the present invention may relate to a method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 10, in which the method includes a step of culturing bacteria of Krasilnikovia sp. The

strain that produces the present enzyme may be either a wild-type strain or a mutant strain (for example, a mutant strain is obtained by UV irradiation).

[0032]

The protein deamidation and protein cross-linking enzyme in the fourth embodiment can be obtained by culturing microorganisms that produce the protein deamidation and protein cross-linking enzyme (i.e., a strain that produces the present enzyme), for example, the bacteria of Longispora sp., such as *Longispora fulva*. That is to say, the present invention may relate to a method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 14, in which the method includes a step of culturing bacteria of Longispora sp. The strain that produces the present enzyme may be either a wild-type strain or a mutant strain (for example, a mutant strain is obtained by UV irradiation).

[0033]

The present enzyme can be prepared using a culture solution and/or a cell mass of microorganisms that produce the present enzyme. The culture conditions or the culture methods are not particularly limited, as long as the present enzyme can be produced thereby. That is to say, the methods or the culture conditions that are adapted to the culture of the used microorganisms can be determined, as appropriate, under conditions in which the present enzyme is produced. Regarding the culture method, either liquid culture or solid culture may be applied, and liquid culture is preferably utilized. Taking the liquid culture as an example, the culture conditions will be described below.

[0034]

The medium is not particularly limited, as long as it is a medium in which the used microorganisms can grow. Examples of the medium that can be used herein may include those to which the following substances are added: carbon sources, such as glucose, sucrose, gentiobiose, soluble starch, glycerin, dextrin, molasses, and organic acids; nitrogen sources, such as ammonium sulfate, ammonium carbonate, ammonium phosphate, ammonium acetate, or gelatin, peptone, yeast extract, corn steep liquor, casein hydrolysate, bran, and meat extract; and further, inorganic salts, such as potassium salts, magnesium salts, sodium salts, phosphates, manganese salts, iron salts and

zinc salts. In order to promote the growth of the used microorganisms, vitamins, amino acids and the like may be added to the medium. The pH of the medium is adjusted to, for example, about 3 to 8, preferably about 4 to 7. The culture temperature is usually about 20°C to 40°C, preferably about 25°C to 35°C, and the microorganisms are cultured for 1 to 20 days, and preferably 3 to 10 days, under aerobic conditions. As a culture method applied herein, for example, a shaking culture method, or an aerobic deep culture method using a jar fermenter, can be utilized.

[0035]

After completion of the culture performed under the above-described conditions, the enzyme of interest is recovered from the culture solution or the culture mass. When the enzyme is recovered from the culture solution, for example, the culture supernatant is filtered, centrifuged, etc. to remove insoluble matters, and thereafter, the resultant is separated and purified by appropriately combining concentration using an ultrafiltration membrane, salting-out such as ammonium sulfate precipitation, dialysis, and various types of chromatography using ion exchange resin, etc., thereby obtaining the present enzyme. On the other hand, when the enzyme is recovered from the cell mass, for example, the cell mass is crushed by a pressure treatment, an ultrasonic treatment, etc., and is then separated and purified in the same manner as that described above, so as to obtain the present enzyme. Besides, the cell mass may be previously recovered from the culture solution by filtration, a centrifugation treatment, etc., and thereafter, the above-described series of steps (crushing, separation, and purification of the cell mass) may be carried out.

[0036]

Also, the present enzyme can be easily prepared by a genetic engineering technique. For example, the present enzyme can be prepared by transforming suitable host cells (for example, *Escherichia coli*) with DNA encoding the present enzyme, and then recovering the protein expressed in the transformant. The recovered protein is appropriately purified, depending on the purpose. As such, if the enzyme of interest is obtained as a recombinant protein, various modifications can be carried out thereon. For example, a DNA encoding the present enzyme and another appropriate DNA are inserted into an identical vector, and a recombinant protein is produced using the vector, so that the present enzyme consisting of a recombinant protein, in which any given peptides or proteins are ligated to each other, can be obtained. In addition, modification may be carried out, so that

addition of a sugar chain and/or a lipid or the processing of the N-terminus or the C-terminus may occur. By performing the aforementioned modification, simplification of the extraction and purification of a recombinant protein, addition of biological functions, etc. can be carried out.
[0037]

Generally, the expression of a gene and the recovery of a gene expression product (the present enzyme) are carried out, utilizing an appropriate host-vector system, as described above. However, a cell-free synthesis system may also be utilized. Herein, the term "cell-free synthesis system (a cell-free transcription system or a cell-free transcription/translation system)" means that, not using living cells, but using a ribosome, a transcription/translation factor or the like derived from living cells (or obtained by a genetic engineering technique), from a nucleic acid (DNA or mRNA) used as a template, mRNA or a protein encoded by the nucleic acid is synthesized *in vitro*. In general, in the cell-free synthesis system, a cell extract obtained by purifying, as necessary, a cell-disintegrated solution is used. The cell extract generally contains a ribosome, various types of factors such as an initiation factor, and various types of enzymes such as tRNA, which are necessary for protein synthesis. When a protein is synthesized, various types of amino acids, energy sources such as ATP or GTP, and other substances necessary for protein synthesis, such as creatine phosphate, are added to the aforementioned cell extract. As a matter of course, upon the synthesis of a protein, a ribosome, various types of factors, and/or various types of enzymes, and the like, which are prepared separately, may also be added to the aforementioned cell extract, as necessary.
[0038]

The development of a transcription/translation system, in which various molecules (factors) necessary for protein synthesis have been reconstructed, has also been reported (Shimizu, Y. et al.: Nature Biotech., 19, 751-755, 2001). In this synthesis system, genes of 31 types of factors composed of: 3 types of initiation factors, 3 types of elongation factors, 4 types of factors associated with termination, 20 types of aminoacyl tRNA synthesis enzymes that allow each amino acid to bind to tRNA, and a methionyl tRNA formyl transfer enzyme, which constitute a protein synthesis system of bacteria, are amplified from an *Escherichia coli* genome, and then, using these amplified products, a protein synthesis system is reconstructed *in vitro*. In the present invention, such a reconstructed synthesis system may be utilized.

[0039]

The term "cell-free transcription/translation system" is exchangeably used with the term "cell-free protein synthesis system," "*in vitro* translation system" or "*in vitro* transcription/translation system." In the *in vitro* translation system, RNA is used as a template to synthesize a protein. As such template RNA, total RNA, mRNA, an *in vitro* transcriptional product or the like is used. On the other hand, in the *in vitro* transcription/translation system, DNA is used as a template. The template DNA should contain a ribosome-binding region, and preferably contains an appropriate terminator sequence. In addition, in the *in vitro* transcription/translation system, in order to promote continuous progression of the transcription reaction and the translation reaction, conditions, in which factors necessary for each reaction are added, are established.

[0040]

The purified enzyme obtained as described above is pulverized, for example, by freeze drying, vacuum drying, or spray drying, so that the enzyme can be provided in the form of powders. At that time, the purified enzyme may be previously dissolved in an acetate buffer, a phosphate buffer, a triethanolamine buffer, a Tris-HCl buffer, or a GOOD buffer. Preferably, an acetate buffer, a phosphate buffer, or a triethanolamine buffer can be used. Besides, examples of the GOOD buffer used herein may include PIPES, MES, and MOPS.

[0041]

The purification degree of the enzyme is not particularly limited. In addition, the final form of the enzyme may be either a liquid or a solid (including powders).

[0042]

The present enzyme catalyzes both the deamidation reaction of the γ-amide group of glutamine residues in proteins and the acyl transfer reaction between the γ-amide group of glutamine residues and the ε-amino group of lysine residues in proteins. The content of the active ingredient (the present enzyme) in the enzyme agent of the present invention is not particularly limited, and for example, the content of the active ingredient can be set or adjusted, so that the protein deamidation activity per gram of the present enzyme agent can become 0.01 U to 5000 U, preferably 0.1 U to 2000 U, and more preferably 1 U to 1000 U. Also, the content of the active ingredient can be set or adjusted, so that the transglutaminase activity per gram of the present enzyme agent can become 0.01

U to 5000 U, preferably 0.1 U to 2000 U, and more preferably 1 U to 1000 U.

[0043]

As a method of measuring the transglutaminase activity, the following method can be adopted. That is, the enzyme is dissolved in 200 mM Tris-HCl (pH 6.0) containing 0.4% cysteine and is then treated at 30°C for 1 hour, and thereafter, the resulting solution is diluted with 200 mM Tris-HCl (pH 6.0) to an appropriate concentration (sample solution). After that, 100 μL of a substrate solution (R-1) obtained by the method described later is added to 10 μL of the sample solution and is mixed, and the obtained mixture is then reacted at 37°C for 10 minutes. Thereafter, 100 μL of a coloring solution (R-2) obtained by the method described later is added to stop the reaction, and at the same time, an Fe complex is formed, and the absorbance at 525 nm is then measured. As a control, the absorbance of an enzyme solution that has been previously heat-inactivated and reacted similarly is measured, and the absorbance difference with the sample solution is determined. Separately, a calibration curve is created using L-glutamic acid-γ-monohydroxamic acid instead of the enzyme solution, and the amount of hydroxamic acid produced is determined from the above-described absorbance difference. The enzyme activity that produces 1 μmol of hydroxamic acid per minute is defined as 1 unit (1 U).

[0044]

(Substrate solution (R-1))

2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 0.70 g of hydroxyammonium hydrochloride, 0.31 g of reduced glutathione, and 1.01 g of Z-Gln-Gly (benzyloxycarbonyl-L-glutaminylglycine) are dissolved in distilled water to a total volume of 100 mL (pH 6.0).

[0045]

(Substrate solution (R-2))

30 mL of 3M hydrochloric acid solution, 30 mL of 12% trichloroacetic acid solution, and 30 mL of 5% iron(III) chloride solution are mixed with one another.

[0046]

As a method for measuring a protein deamidation activity, the following method using a synthetic substrate can be employed. That is, 0.1 mL of a sample solution containing a protein deamidase is added to 1 mL of a 0.2 M phosphate buffer (pH 6.5) containing 30 mM Z-Gln-Gly, the

obtained mixture is then left at 37°C for 10 minutes, and 1 mL of a 0.4 M TCA (trichloroacetic acid) solution is then added to stop the reaction. As a blank, 1 mL of a 0.4 M TCA solution is added to 1 mL of a 0.2 M phosphate buffer (pH 6.5) containing 30 mM Z-Gln-Gly, and 0.1 mL of a sample solution containing a protein deamidase is further added thereto, followed by leaving the obtained mixture at 37°C for 10 minutes.

[0047]

"Ammonia Test Wako" (FUJIFILM Wako Pure Chemical Corporation) was used for the solution obtained above, and the amount of ammonia generated in the reaction solution was measured. The ammonia concentration in the reaction solution is determined from a calibration curve showing the relationship between the ammonia concentration and the absorbance (630 nm) prepared using an ammonia standard solution (ammonium chloride).

[0048]

The activity of the protein deamidase is calculated using the formula shown below, in which the amount of enzyme that produces 1 μmol of ammonia per minute is defined as 1 unit (1 U). In the formula, the amount of the reaction solution is 2.1, the amount of the enzyme solution is 0.1, and Df is the dilution magnification of the enzyme solution. Also, 17.03 is the molecular weight of ammonia.

[0049]

[Expression 1]

Protein deamidase activity (U/mL)

= Ammonia concentration (mg/L) in reaction solution x (1 / 17.03) x (reaction solution amount / enzyme solution amount) x (1/10) x Df

[0050]

On the other hand, when the protein deamidation activity with respect to the protein substrate is measured, and when the ratio between the protein deamidation activity and the transglutaminase activity is calculated, it can be calculated from the ratio between the amount of deamidated insulin B chain produced and the decreased amount of insulin B chain when the insulin

B chain is used as a substrate. Details of the measurement conditions, measurement procedures, and the like are shown in the Examples described later.

[0051]

The protein deamidation activity in the enzyme agent of the present invention is not particularly limited, and for example, the lower limit value of the deamidation percentage in the evaluation method using insulin B chain is 4% or more, 5% or more, or 7% or more, and it is preferably 10% or more, and more preferably 15% or more. On the other hand, the upper limit value of the deamidation percentage is 99% or less, 90% or less, 80% or less, 70% or less, 60% or less, 50% or less, or 40% or less, and it is preferably 30% or less, and more preferably 20% or less.

[0052]

The transglutaminase activity in the enzyme agent of the present invention is not particularly limited, and for example, the lower limit value of the cross-linking percentage in the evaluation method using insulin B chain is 10% or more, 20% or more, 30% or more, or 40% or more, and it is preferably 50% or more, and more preferably 60% or more. The upper limit value of the cross-linking percentage is 95% or less, 92% or less, or 90% or less, and it is preferably 88% or less, and more preferably 85% or less.

[0053]

The ratio between the protein deamidation activity and the transglutaminase activity in the present enzyme agent is not particularly limited, and for example, the "protein deamidation activity/transglutaminase activity" in the evaluation method using insulin B chain may be 0.04 or more, 0.05 or more, 0.06 or more, 0.07 or more, 0.08 or more, 0.09 or more, 0.10 or more, 0.11 or more, 0.12 or more, 0.13 or more, 0.14 or more, 0.15 or more, 0.16 or more, 0.17 or more, 0.18 or more, 0.19 or more, or 0.20 or more.

[0054]

Moreover, as described above, the protein deamidation activity and the transglutaminase activity of the enzyme agent of the present invention can also be evaluated by analyzing the reaction stop solution using HPLC, after the reaction has been carried out with the synthetic substrate, and detecting the peak transition. Details of the measurement conditions and measurement procedures are shown in the Examples described later.

[0055]

The enzyme agent of the present invention may also contain an excipient, a buffer agent, a suspending agent, a stabilizer, a preservative, an antiseptic, a normal saline, various types of proteins, degradation products of various types of proteins, various types of extracts, various types of salts, various types of antioxidants, cysteine, glutathione, sodium glutamate, sodium inosinate, sodium guanylate, calcined shell calcium, silicon dioxide, and the like, as well as the active ingredient (the protein deamidation and protein cross-linking enzyme of the present invention). Examples of the excipient that can be used herein may include starch, dextrin, maltose, trehalose, lactose, D-glucose, sorbitol, D-mannitol, white sugar, and glycerol. Examples of the buffer agent that can be used herein may include phosphate, citrate, and acetate. Examples of the stabilizer that can be used herein may include propylene glycol and ascorbic acid. Examples of the preservative that can be used herein may include phenol, benzalkonium chloride, benzyl alcohol, chlorobutanol, and methylparaben. Examples of the antiseptic that can be used herein may include ethanol, benzalkonium chloride, paraoxybenzoic acid, and chlorobutanol. Examples of the protein used herein may include a soybean protein, a wheat protein, a corn protein, a milk protein, and an animal-derived protein. Examples of the extracts used herein may include a meat extract, a plant extract, and a yeast extract. Examples of the salts used herein may include chloride, phosphate, polyphosphate, pyrophosphate, citrate, lactate, and carbonate. Examples of the antioxidant used herein may include L-ascorbate and sodium hydrogen sulfite. The shape of the enzyme agent of the present invention is not particularly limited, and it is, for example, powders, granules, a liquid, a capsule, or the like.

[0056]

2. Gene

In the first embodiment, the gene encoding the present enzyme consists of DNA encoding a protein containing the amino acid sequence as set forth in SEQ ID NO: 1. Specific examples of the present aspect may include DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 3, and DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 4. The DNA of SEQ ID NO: 3 encodes only the amino acid sequence (SEQ ID NO: 1) of a mature form, whereas the DNA of SEQ ID NO: 4 encodes a signal peptide and a pro-sequence, in addition to the mature form (the amino acid sequence as set forth in SEQ ID NO: 1).

[0057]

In the second embodiment, the gene encoding the present enzyme consists of DNA encoding a protein containing the amino acid sequence as set forth in SEQ ID NO: 6. Specific examples of the present aspect may include DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 8, and DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 9. The DNA of SEQ ID NO: 8 encodes only the amino acid sequence (SEQ ID NO: 6) of a mature form, whereas the DNA of SEQ ID NO: 9 encodes a signal peptide and a pro-sequence, in addition to the mature form (the amino acid sequence as set forth in SEQ ID NO: 6).

[0058]

In the third embodiment, the gene encoding the present enzyme consists of DNA encoding a protein containing the amino acid sequence as set forth in SEQ ID NO: 10. Specific examples of the present aspect may include DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 12, and DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 13. The DNA of SEQ ID NO: 12 encodes only the amino acid sequence (SEQ ID NO: 10) of a mature form, whereas the DNA of SEQ ID NO: 13 encodes a signal peptide and a pro-sequence, in addition to the mature form (the amino acid sequence as set forth in SEQ ID NO: 10).

[0059]

In the fourth embodiment, the gene encoding the present enzyme consists of DNA encoding a protein containing the amino acid sequence as set forth in SEQ ID NO: 14. Specific examples of the present aspect may include DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 16, and DNA consisting of the nucleotide sequence as set forth in SEQ ID NO: 17. The DNA of SEQ ID NO: 16 encodes only the amino acid sequence (SEQ ID NO: 14) of a mature form, whereas the DNA of SEQ ID NO: 17 encodes a signal peptide and a pro-sequence, in addition to the mature form (the amino acid sequence as set forth in SEQ ID NO: 14).

[0060]

The gene encoding the present enzyme is typically utilized in preparation of the present enzyme. According to a genetic engineering preparation method using the gene encoding the present enzyme, it is possible to obtain the present enzyme in a more homogeneous state. In addition, it is said that this method is a method preferably used in the case of preparing a large

amount of the present enzyme. Besides, the intended use of the gene encoding the present enzyme is not limited to the preparation of the present enzyme. For example, the present nucleic acid can also be used as an experimental tool for elucidating the action mechanism of the present enzyme, or as a tool for designing or preparing a mutant (a modified body) of the present enzyme.

[0061]

In the present description, the phrase "the gene encoding the present enzyme" means a nucleic acid for providing the present enzyme, when the nucleic acid is allowed to express. Thus, the gene encoding the present enzyme includes not only a nucleic acid having a nucleotide sequence corresponding to the amino acid sequence of the present enzyme, but also a nucleic acid formed by adding a sequence that does not encode the amino acid sequence to the aforementioned nucleic acid. Moreover, codon degeneracy is also taken into consideration.

[0062]

The nucleic acid can be prepared in an isolated state according to a standard genetic engineering technique, a molecular biological technique, a biochemical technique, chemical synthesis, a PCR method (for example, overlap PCR), or a combination thereof, with reference to the present description or the sequence information disclosed in the sequence listing attached herewith.

[0063]

There may be used a nucleic acid, in which when the nucleic acid is compared with the nucleotide sequence of the gene encoding the present enzyme, the function of a protein encoded thereby is equivalent, but the nucleotide sequence thereof is partially different from the nucleotide sequence of the gene encoding the present enzyme (which is hereinafter also referred to as an "equivalent nucleic acid," and the nucleotide sequence of the equivalent nucleic acid is also referred to as an "equivalent nucleotide sequence"). An example of such an equivalent nucleic acid may be DNA that consists of a nucleotide sequence having a substitution, deletion, insertion, addition or inversion of one or more nucleotides, based on the nucleotide sequence of the nucleic acid encoding the present enzyme, and encodes a protein having an enzyme activity characteristic of the present enzyme (i.e. protein deamidation activity and transglutaminase activity). Substitution, deletion, etc. of a nucleotide(s) may occur in multiple sites. The term "multiple" is used herein to mean for example, 2 to 40 nucleotides, preferably 2 to 20 nucleotides, and more preferably 2 to 10 nucleotides,

although the number of nucleotides is different depending on the positions or types of amino acid residues in the three-dimensional structure of a protein encoded by the nucleic acid. The equivalent nucleic acid has a sequence identity of, for example, 50% or more, 60% or more, 65% or more, 70% or more, 80% or more, 81% or more, 82% or more, 83% or more, 84% or more, 85% or more, 86% or more, 87% or more, 88% or more, 89% or more, 90% or more, 91% or more, or 92% or more, preferably 93% or more, more preferably 94% or more, even more preferably 95% or more, further preferably 96% or more, even further preferably 97% or more, still further preferably 98% or more, and most preferably 99% or more, with respect to the nucleotide sequence serving as a reference (SEQ ID NO: 3, 4, 8, 9, 12, 13, 16, or 17).

[0064]

Such an equivalent nucleic acid as described above can be obtained by, for example, a restriction enzyme treatment, a treatment with exonuclease, DNA ligase, etc., introduction of a mutation according to a site-directed mutagenesis (Molecular Cloning, Third Edition, Chapter 13, Cold Spring Harbor Laboratory Press, New York) or a random mutation introduction method (Molecular Cloning, Third Edition, Chapter 13, Cold Spring Harbor Laboratory Press, New York), etc. Moreover, the equivalent nucleic acid can also be obtained by other methods such as ultraviolet irradiation.

[0065]

There may be used a nucleic acid having a nucleotide sequence complementary to the nucleotide sequence of the gene that encodes the present enzyme. There may also be used a nucleic acid having a nucleotide sequence that has a sequence identity of, at least, about 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, to the nucleotide sequence of the gene that encodes the present enzyme, or to a nucleotide sequence complementary to the nucleotide sequence of the gene that encodes the present enzyme.

[0066]

There may also be used a nucleic acid having a nucleotide sequence that hybridizes, under stringent conditions, to a nucleotide sequence complementary to the nucleotide sequence of the gene that encodes the present enzyme or complementary to a nucleotide sequence equivalent thereto.

The term "stringent conditions" is used herein to mean conditions under which, what is called, a specific hybrid is formed, but a non-specific hybrid is not formed. Such stringent conditions are known to those skilled in the art, and can be determined, for example, with reference to Molecular Cloning (Third Edition, Cold Spring Harbor Laboratory Press, New York) or Current protocols in molecular biology (edited by Frederick M. Ausubel et al., 1987). The stringent conditions may be, for example, conditions in which incubation is performed at about 50℃, using a hybridization solution (50% formamide, 10 x SSC (0.15 M NaCl, 15 mM sodium citrate, pH 7.0), 5 x Denhardt solution, 1% SDS, 10% dextran sulfate, 10 μg/ml denatured salmon sperm DNA, and 50 mM phosphate buffer (pH 7.5)), and thereafter, washing is performed at about 65℃, using 0.1 x SSC and 0.1% SDS. More preferred stringent conditions may be, for example, conditions of using, as a hybridization solution, 50% formamide and 5 x SSC (0.15 M NaCl, 15 mM sodium citrate, pH 7.0), 1 x Denhardt solution, 1% SDS, 10% dextran sulfate, 10 μg/ml denatured salmon sperm DNA, 50 mM phosphate buffer (pH 7.5)).

[0067]

There may be used recombinant DNA containing the gene that encodes the present enzyme. The recombinant DNA is provided in the form of, for example, a vector. The term "vector" is used in the present description to mean a nucleic acid molecule capable of transporting a nucleic acid inserted in the nucleic acid molecule into a target such as a cell.

[0068]

Depending on intended purpose (cloning or the expression of a protein), or taking into consideration of the type of a host cell, a suitable vector is selected. Examples of the vector having *Escherichia coli* as a host may include M13 phage or a modified body thereof, λ phage or a modified body thereof, and pBR322 or a modified body thereof (pB325, pAT153, pUC8, etc.). Examples of the vector having yeast as a host may include pYepSec1, pMFa, and pYES2. Examples of the vector having an insect cell as a host may include pAc and pVL. Examples of the vector having a mammalian cell as a host may include pCDM8 and pMT2PC.

[0069]

The vector is preferably an expression vector. The term "expression vector" means a vector that is capable of introducing a nucleic acid inserted in the vector into a cell of interest (a host

cell) and that is also capable of allowing the nucleic acid to express in the host cell. The expression vector usually contains a promoter sequence necessary for the expression of the inserted nucleic acid, an enhancer sequence for promoting the expression, and the like. An expression vector containing a selective marker can also be used. In the case of using such an expression vector, the presence or absence of the introduction of the expression vector (and the degree thereof) can be confirmed by utilizing the selective marker.

[0070]

Insertion of the nucleic acid into the vector, insertion of a selective marker (if necessary), insertion of a promoter (if necessary), and the like can be carried out by using a standard recombinant DNA technique (for example, a publicly known method using restriction enzyme and DNA ligase, which can refer to Molecular Cloning, Third Edition, 1.84, Cold Spring Harbor Laboratory Press, New York).

[0071]

In terms of the ease of handling, microorganisms such as *Escherichia coli*, Filamentous fungi (*Aspergillus oryzae*), Actinomycetes (Streptomyces sp., *Streptomyces lividans*, and *Streptomyces mobaraensis*), *Bacillus subtilis*, and budding yeast (*Saccharomyces cerevisiae*) are preferably used as host cells. However, any host cells can be utilized, as long as they are capable of replicating recombinant DNA and expressing the gene of the present enzyme. In the case of utilizing a T7 promoter, the used *Escherichia coli* may be, for example, the *Escherichia coli* BL21(DE3)pLysS. In the case of not using a T7 promoter, the used *Escherichia coli* may be, for example, the *Escherichia coli* JM109. In addition, examples of the budding yeast may include the budding yeast SHY2, the budding yeast AH22, and the budding yeast INVSc1 (Invitrogen).

[0072]

A microorganism containing the recombinant DNA (i.e. a transformant) can be used. The microorganism can be obtained by transfection or transformation using the above-described vector of the present invention. Such transfection or transformation can be carried out, for example, by a calcium chloride method (Journal of Molecular Biology (J. Mol. Biol.), Vol. 53, p. 159 (1970)), a Hanahan method (J. Mol. Biol., Vol. 166, p. 557 (1983)), an SEM method (Gene, Vol. 96, p. 23 (1990)), a method of Chung, et al. (Proceedings of the National Academy of Sciences of the USA.

Vol. 86, p. 2172 (1989)), a calcium phosphate coprecipitation method, electroporation (Potter, H. et al., Proc. Natl. Acad. Sci. U.S.A. 81, 7161-7165 (1984)), lipofectin (Felgner, P. L. et al., Proc. Natl. Acad. Sci. U.S.A. 84, 7413-7417 (1984)), a conjugative transfer method, and the like. The above-described microorganism can be utilized to produce the present enzyme.

[0073]

3. Intended use of enzyme agent

The present invention further relates to an intended use of the enzyme agent of the present invention.

That is to say, a protein or a peptide can be modified (e.g., the improvement of water retentivity, oil retentivity, emulsifiability, emulsification stability, foaming ability, foam stability, protein solubility, moldability, etc.) by allowing the enzyme agent of the present invention to act on a substrate (a protein or a peptide). Specifically, by allowing the enzyme agent of the present invention to act on a protein or a peptide containing glutamine residues and lysine residues, deamidation of the glutamine residues of the protein or the cross-linking of the glutamine residues and the lysine residues is carried out, so that the stickiness of the protein or the peptide after the enzyme treatment can be improved. That is, the present invention may relate to a method for deamidating and cross-linking a protein or a peptide, including allowing an enzyme agent for protein deamidation and protein cross-linking to act on the protein or the peptide. Among others, the protein or the peptide is preferably contained in protein-containing food product and beverage, and the present invention preferably relates to a method for deamidating and cross-linking protein-containing food product and beverage, including allowing an enzyme agent for protein deamidation and protein cross-linking to act on protein-containing food product and beverage.

[0074]

The present invention may relate to a method for producing food product and beverage, including allowing an enzyme agent for protein deamidation and protein cross-linking to act on protein-containing food product and beverage. For example, by allowing the enzyme agent of the present invention to act on food product and beverage or a food product and beverage material (protein-containing food product and beverage, etc.), which contains a protein or a peptide, food product and beverage or a food product and beverage material (deamidated and cross-linked protein-

containing food product and beverage, etc.), which contains a deamidated and cross-linked protein, can be produced. By allowing the enzyme agent of the present invention to act on food product and beverage, the quality of food product and beverage can be improved. According to the present invention, food product and beverage treated with the enzyme agent of the present invention (deamidated and cross-linked protein-containing food product and beverage) is provided. Moreover, the enzyme agent of the present invention can also be used for production of industrial materials and pharmaceutical raw materials, other than food products and beverages or food product and beverage materials (protein-containing food products and beverages, etc.).

[0075]

Specific examples of the improvement of the quality of protein-containing food product and beverage may include, but are not particularly limited, intended uses for improving the stickiness of animal proteins or plant proteins (e.g., intended uses for production of meat products (hamburgers, restructured meat, pork cutlets, sausages, steaks, ham, meatballs, etc.), seafood products (seafood, fish roe, *kamaboko*, tuna nuggets, etc.), processed egg products (steamed egg custard, mayonnaise, etc.), grains and processed grain products (rice, wheat, buckwheat, grains, corn, pasta, and *udon*), bread and confectionery (pancakes, cookies, etc.), dairy products (milk, cheese, yogurt, cream, ice cream, butter, etc.), plant-based milk, plant-based cheese, plant-based yogurt, animal gelatin, plant-based gel, animal gel, *tofu*, meat substitutes, dressings, etc.).

[0076]

The method for producing food product and beverage using the present enzyme agent is not particularly limited, and it is, for example, a method including the following step (a). Furthermore, the method may also include a step (b) and/or a step (c), after the step (a):

(a) a step of allowing the present enzyme agent to act on a food product or a food product material that contains a protein (protein-containing food product and beverage),

(b) a step of recovering the food product or the food product material, which contains the deamidated and cross-linked protein, and

(c) a step of deactivating or removing the present enzyme contained in the obtained enzyme-treated food product or enzyme-treated food product material.

Examples

[0077]

Example 1: Preparation of enzyme having protein deamidation activity and transglutaminase activity, derived from *Longimycelium tulufanense*

A cell mass of *Longimycelium tulufanense* (obtained from the National Institute of Technology and Evaluation (NITE)) (NBRC107726) was cultured in Trypto-Sawyer Broth Medium (manufactured by Nissui Pharmaceutical Co., Ltd.) at 37°C for 4 days, and the supernatant was then recovered. The obtained supernatant was dialyzed with 20 mM phosphate buffer (pH 6) according to an ordinary method. The dialyzed sample was subjected to ion exchange chromatography using a cation exchange column (SP Sepharose FF, manufactured by GE HealthCare) equilibrated with the same buffer as described above. After desalting and concentrating the active fraction, the resultant was further subjected to gel filtration using Superdex 200 HR to obtain the present enzyme sample.

[0078]

The amino acid sequences and nucleotide sequences of the present enzyme are shown below.

Amino acid sequence (only mature body sequence) (SEQ ID NO: 1)

ATSLPPIAPPLPRGVQSKSWSVPDYIAAWEKQHGRPMTAEERYHLARGCIGVTVVNLDRE
DAPNPPLNLSFGTYQRAMEVQAALNEIVATRPSPREYAEQVRKHPALQGVQNLVRAFPT
FIDPANLHAAIFSKRFYSKQDPNWTDEQAAEMYRPNPRTGQVDMSTYRYRARPGYVNFD
YGWYDEQTNNWWHANHAEPGMKVYQSTLRYYSRPLLDFDEQVFTVAFARVA

[0079]

Amino acid sequence (full length) * The underlined part indicates the mature body sequence. (SEQ ID NO: 2)

MKKWLPRALVALFVLLGLPAGLAGTAHAAVVAHAAV<u>ATSLPPIAPPLPRGVQSKSWSVP
DYIAAWEKQHGRPMTAEERYHLARGCIGVTVVNLDREDAPNPPLNLSFGTYQRAMEVQ
AALNEIVATRPSPREYAEQVRKHPALQGVQNLVRAFPTFIDPANLHAAIFSKRFYSKQDPN
WTDEQAAEMYRPNPRTGQVDMSTYRYRARPGYVNFDYGWYDEQTNNWWHANHAEP
GMKVYQSTLRYYSRPLLDFDEQVFTVAFARVA</u>

[0080]

Nucleotide sequence (mature body) (SEQ ID NO: 3)

gccacgtcgctgccaccgatcgcaccgccgcttccccggggcgtgcagagcaagagctggtcggtgccggactacattgccgcctgggaga
agcaacacggtaggccgatgacggccgaggagcggtaccacctcgcccggggctgcatcggcgtcaccgtggtcaacctcgaccgggagg
acgcgccgaacccgccgctcaacctgtcgttcggcacttaccagcgggccatggaagtgcaggccgcgctgaacgagatcgtcgcgacccg
gccgtcgccgcgggagtacgccgagcaggtgcgcaagcacccggcgctgcagggcgtgcagaacctggtgcgggcgttccccacgttcat
cgacccggcgaacctgcacgccgccatcttctccaagcggttctactcgaagcaggacccgaactggaccgacgagcaggcggccgagatg
taccggccgaatccgcggaccggccaggtcgacatgagcacctaccgctaccgggcgcggccgggttacgtgaacttcgactacggttggta
cgacgagcagacgaacaactggtggcacgccaaccacgcggagccgggcatgaaggtctaccagagcacgttgcggtactactcccggcc
gctgctggatttcgacgagcaggttttcaccgtggcgttcgcgcgggtcgcctga

[0081]

Nucleotide sequence (full length) (SEQ ID NO: 4)

gtgaagaagtggttgccgcgcgccctggtcgcgctgttcgtgctcctggggttacccgccggcctggccggaacggcacatgccgcggtcgtc
gcgcacgccgccgtcgccacgtcgctgccaccgatcgcaccgccgcttccccggggcgtgcagagcaagagctggtcggtgccggactaca
ttgccgcctgggagaagcaacacggtaggccgatgacggccgaggagcggtaccacctcgcccggggctgcatcggcgtcaccgtggtcaa
cctcgaccgggaggacgcgccgaacccgccgctcaacctgtcgttcggcacttaccagcgggccatggaagtgcaggccgcgctgaacgag
atcgtcgcgacccggccgtcgccgcgggagtacgccgagcaggtgcgcaagcacccggcgctgcagggcgtgcagaacctggtgcgggc
gttccccacgttcatcgacccggcgaacctgcacgccgccatcttctccaagcggttctactcgaagcaggacccgaactggaccgacgagca
ggcggccgagatgtaccggccgaatccgcggaccggccaggtcgacatgagcacctaccgctaccgggcgcggccgggttacgtgaacttc
gactacggttggtacgacgagcagacgaacaactggtggcacgccaaccacgcggagccgggcatgaaggtctaccagagcacgttgcggt
actactcccggccgctgctggatttcgacgagcaggttttcaccgtggcgttcgcgcgggtcgcctga

[0082]

Example 2: Confirmation of protein-modifying effect

The protein-modifying effect (the improvement of stickiness) when using a casein solution as a substrate was confirmed using the present enzyme sample.

(Method)

Milk casein (Casein, Bovine Milk, Carbohydrate and Fatty Acid Free, Merck KGaA) was dissolved in water to prepare an 11.1% (w/v) casein solution. To 9 mL of the 11.1% (w/v) casein solution, an enzyme solution was added at a ratio of 0.25 mg and 1 mg per gram of the casein protein, and the obtained solution was then made up to 10 mL with a 50 mM sodium phosphate

buffer (pH 7.0). Thereafter, the entire amount of reaction solution was poured into a Φ56 mm plastic petri dish, and was then incubated at 40°C for 2 hours, followed by leaving at rest overnight (12 to 16 hours) at 4°C to obtain an enzyme-treated casein protein. For comparison, the same procedures as described above were performed using Streptomyces-derived transglutaminase (manufactured by Amano Enzyme Inc.), instead of the present enzyme.

< Evaluation of stickiness >

In order to evaluate the physical properties of the enzyme-treated casein protein, a rheometer was used. Specifically, a metal plunger having a diameter of 20 mm was used to push the sample forward at a rate of 60 mm/min, with a sample advance of 4 mm. Thereafter, the distance over which the stress (adhesion) generated when the plunger separated from the sample persisted was measured.

[0083]

(Results)

The results are shown in Figure 1 and Table 1.

[Table 1]

Amount of enzyme added	Stickiness (mm)	
	The present enzyme	Streptomyces-derived transglutaminase
0.25 mg	14	5
1 mg	5	1

[0084]

(Consideration)

In the results shown in Figure 1 and Table 1, it could be confirmed that the present enzyme having both a protein deamidation activity and a transglutaminase activity showed a significant increase in stickiness, compared to existing transglutaminases.

[0085]

Example 3: Evaluation of protein deamidation activity and transglutaminase activity on protein substrate

(Method)

5 mg of Insulin B chain (insulin B chain, oxidized form, derived from bovine pancreas, Sigma-Aldrich, sequence: Phe-Val-Asn-Gln-His-Leu-Cys(SO_3H)-Gly-Ser-His-Leu-Val-Glu-Ala-

Leu-Tyr-Leu-Val-Cys(SO_3H)-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala (SEQ ID NO: 5)) was dissolved in 2 mL of ultrapure water to prepare a substrate solution. Thereafter, 20 μL of a 1 mg/mL purified enzyme solution and a 0.2 M potassium dihydrogen phosphate/disodium hydrogen phosphate buffer (pH 6.5) were added to 90 μL of the substrate solution to a total volume of 180 μL, so as to prepare a reaction solution. The reaction solution was heated to 40°C in an incubator and was then left at rest for 1 hour. Thereafter, 20 μL of 1 N HCl was added to the reaction solution to stop the reaction. The described reaction stop solution was analyzed using HPLC. Deamidation activity and transglutaminase activity were detected by the peak transition from the insulin B chain to the deamidated insulin B chain. As a comparative example, deamidation activity and transglutaminase activity were similarly detected using Streptomyces-derived transglutaminase (manufactured by Amano Enzyme Inc.).

(Protein deamidation activity)

The amount of the deamidated insulin B chain produced was calculated from the peak areas of the insulin B chain and the deamidated insulin B chain that were calculated from the chromatogram, and the protein deamidation activity was then evaluated using the deamidation percentage (amount of deamidated insulin B chain produced ÷ amount of substrate insulin B chain x 100).

(Transglutaminase activity)

From each peak area calculated from the chromatogram, the amount of residual insulin B chain and the amount of deamidated insulin B chain produced were calculated and were then evaluated using the cross-linking percentage ((100 - (amount of residual insulin B chain ÷ amount of substrate insulin B chain x 100) - deamidation percentage)).

From the ratio between the deamidation percentage and cross-linking percentage calculated using the above-described methods, the ratio between the protein deamidation activity and the transglutaminase activity was calculated.

[0086]

(Results)

The results are shown in Table 2.

[Table 2]

	Comparative Example	Example
Enzyme used	Streptomyces-derived transglutaminase	The present enzyme
Protein deamidation activity percentage (%)	3.5	18.9
Transglutaminase activity percentage (%)	96.5	81.1
Protein deamidation activity/transglutaminase activity	0.036	0.23

[0087]

Example 4: Confirmation of protein-modifying effect (production of processed protein-containing dry composition with modified physical properties)

(Method)

The protein materials shown in the table below were dissolved or suspended in water to prepare a protein solution having a final concentration of 5% by mass.

[Table 3]

Product Name	Origin	Protein Content (% by mass)	Manufacturer
SoyPro	Soybeans	50%	J-OIL MILLS.
NUTRALYS F85M	Peas	85%	Roquette Japan
Mung bean protein	Mung beans	80%	Ingredion
Fava bean protein	Broad beans	80%	Ingredion
Lentil protein	Lentils	50%	Organo Corporation
Chickpea protein	Chickpeas	84%	Organo Corporation
Oat protein	Oats	75%	Organo Corporation
Rice protein	Rice	85%	Organo Corporation
Whole grain barley flour	Barley	6.7%	Tomizawa Shouten
Whole grain sorghum flour	Sorghum	9.6%	Tomizawa Shouten
Whole grain rye flour	Rye	8.5%	Tomizawa Shouten
Corn gluten meal asia	Corn	-	Roquette Japan
Almond protein	Almonds	85%	Bioactives Japan
Peanut powder	Peanuts	85%	Bioactives Japan
Walnut powder	Walnuts	14.6%	Tomizawa Shouten
Hazelnut powder	Hazelnuts	13%	Tomizawa Shouten
Cashew nut powder	Cashews	20%	Minoya Co., Ltd.
Coconut powder	Coconuts	7%	Alishan
Pistachio powder	Pistachios	17%	Minoya Co., Ltd.
Chia seed protein	Chia seeds	83%	Organo Corporation
Hemp protein	Industrial hemp*1	85%	Bioactives Japan
Casein from bovine milk	Milk casein	94%	Merck
Pork powder	Cattle meal	70%	Unknown
Beef powder	Pork meal	70%	Unknown
Chicken powder	Poultry meal	70%	Unknown
Egg albumin	Egg albumin	100%	Wako Pure Chemical Industries

*1:"Industrial hemp" refers to hemp used for industrial purposes, and specifically means varieties that do not contain THC (tetrahydrocannabinol), a substance that alters perception, or that have a low THC concentration.

[0088]

The enzyme prepared in Example 1 was added to the protein solution at a ratio of 1 mg of the enzyme per 1 g of each protein material shown in the above table, and the obtained mixture was then incubated at 50°C for 16 hours. Thereafter, the enzyme was inactivated by a heat treatment at 100°C for 5 minutes. The resulting enzyme-treated protein solution was freeze-dried to obtain a processed protein-containing dry composition (treated with the present enzyme). Separately, a protein-containing dry composition that had not undergone an enzyme treatment (untreated with enzymes) was also prepared. The effects of modifying physical properties were evaluated for both the processed protein-containing dry composition and the protein-containing dry composition (untreated with enzymes) from the viewpoints A through G described below. Additionally, for some protein materials, a processed protein-containing dry composition treated with Streptomyces-

derived transglutaminase was also prepared, and was subjected to the same evaluation as described above.

[0089]

(A: Water retentivity evaluation)

An aqueous suspension was prepared by suspending 0.1 g of the processed protein-containing dry composition in 1 g of deionized water and then vortexing the mixture for 30 seconds. After the aqueous suspension had been left at rest for 30 minutes, it was centrifuged at 2,000 × g for 10 minutes, and the supernatant was then recovered. The mass of the recovered supernatant was measured, and the mass of water absorbed by the protein was then calculated by subtracting this measured value from 1 g (the mass of the deionized water used for the suspension). Furthermore, the mass of water absorbed per 1 g of the processed protein-containing dry composition was calculated, and the obtained value was designated as "water retentivity." The water retentivity of the enzyme-untreated protein-containing dry composition was also calculated in the same manner as described above. The relative value of the water retentivity of the processed protein-containing dry composition, when the water retentivity of the enzyme-untreated protein-containing dry composition was set at 1, was determined as an evaluation value for water retentivity. In addition, for some protein materials, the relative value of the water retentivity of the processed protein-containing dry composition, when the water retentivity of the processed protein-containing dry composition treated with Streptomyces-derived transglutaminase was set at 1, was determined as an evaluation value for water retentivity.

[0090]

(Results)

The results are shown in the following tables.

[Table 4]

	Origin		Water retentivity
Pulses	Soybeans	Untreated with enzymes	1.00
		The present enzyme	1.37
	Peas	Untreated with enzymes	1.00
		The present enzyme	1.80
	Fava beans	Untreated with enzymes	1.00
		The present enzyme	1.13
	Lentils	Untreated with enzymes	1.00
		The present enzyme	1.80
	Chickpeas	Untreated with enzymes	1.00
		The present enzyme	1.15
Grains	Rye	Untreated with enzymes	1.00
		The present enzyme	1.13
	Corn	Untreated with enzymes	1.00
		The present enzyme	1.04
	Sorghum	Untreated with enzymes	1.00
		The present enzyme	1.06
Nuts	Cashew nuts	Untreated with enzymes	1.00
		The present enzyme	1.56
	Almonds	Untreated with enzymes	1.00
		The present enzyme	1.39
	Peanuts	Untreated with enzymes	1.00
		The present enzyme	1.57
	Industrial hemp	Untreated with enzymes	1.00
		The present enzyme	1.15

[Table 5]

	Origin		Water retentivity
Pulses	Fava beans	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.00
		The present enzyme	1.13
	Lentils	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.33
		The present enzyme	1.80
	Chickpeas	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.81
		The present enzyme	1.15
Grains	Rye	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.96
		The present enzyme	1.13
	Sorghum	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.03
		The present enzyme	1.06
Nuts	Cashew nuts	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.33
		The present enzyme	1.56
	Almonds	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.13
		The present enzyme	1.39
	Industrial hemp	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.10
		The present enzyme	1.15

[Table 6]

	Origin		Water retentivity
Grains	Rice	Streptomyces transglutaminase	1.00
		The present enzyme	1.17
Nuts	Hazelnuts	Streptomyces transglutaminase	1.00
		The present enzyme	1.12
	Coconuts	Streptomyces transglutaminase	1.00
		The present enzyme	1.31

[0091]

Compared to samples untreated with enzymes, the use of the present enzyme resulted in the improvement of water retentivity in soybeans, peas, fava beans, lentils, chickpeas, rye, corn, sorghum, cashew nuts, almonds, peanuts, and industrial hemp. Among others, for fava beans, lentils, chickpeas, rye, sorghum, cashew nuts, almonds, and industrial hemp, the effect of improving water retentivity by the present enzyme was higher than that by the use of Streptomyces-derived transglutaminase.

[0092]

(B: Oil retentivity evaluation)

An oil suspension was prepared by suspending 0.1 g of the processed protein-containing dry composition in 1 g of canola oil and then vortexing the mixture for 30 seconds. After the oil suspension had been left at rest for 30 minutes, it was centrifuged at 2,000 × g for 10 minutes, and the supernatant was then recovered. The mass of the recovered supernatant was measured, and the mass of oil absorbed by the protein was then calculated by subtracting this measured value from 1 g (the mass of the canola oil used for the suspension). Furthermore, the mass of oil absorbed per 1 g of the processed protein-containing dry composition was calculated, and the obtained value was

designated as "oil retentivity." The oil retentivity of the enzyme-untreated protein-containing dry composition was also calculated in the same manner as described above. The relative value of the oil retentivity of the processed protein-containing dry composition, when the oil retentivity of the enzyme-untreated protein-containing dry composition was set at 1, was determined as an evaluation value for oil retentivity. In addition, for some protein materials, the relative value of the oil retentivity of the processed protein-containing dry composition, when the oil retentivity of the processed protein-containing dry composition treated with Streptomyces-derived transglutaminase was set at 1, was determined as an evaluation value for oil retentivity.

[0093]

(Results)

The results are shown in the following tables.

[Table 7]

	Origin		Oil retentivity
Pulses	Lentils	Untreated with enzymes	1.00
		The present enzyme	1.12
Grains	Corn	Untreated with enzymes	1.00
		The present enzyme	1.04
Nuts	Cashew nuts	Untreated with enzymes	1.00
		The present enzyme	1.13
	Almonds	Untreated with enzymes	1.00
		The present enzyme	1.03
	Walnuts	Untreated with enzymes	1.00
		The present enzyme	1.02
	Coconuts	Untreated with enzymes	1.00
		The present enzyme	1.06
	Industrial hemp	Untreated with enzymes	1.00
		The present enzyme	1.05

[Table 8]

	Origin		Oil retentivity
Pulses	Lentils	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.98
		The present enzyme	1.12
Grains	Corn	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.98
		The present enzyme	1.04
Nuts	Cashew nuts	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.97
		The present enzyme	1.13
	Almonds	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.94
		The present enzyme	1.03
	Walnuts	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.88
		The present enzyme	1.02

[Table 9]

	Origin		Oil retentivity
Pulses	Mung beans	Streptomyces transglutaminase	1.00
		The present enzyme	1.79
	Chickpeas	Streptomyces transglutaminase	1.00
		The present enzyme	1.23
Grains	Rice	Streptomyces transglutaminase	1.00
		The present enzyme	1.18
	Rye	Streptomyces transglutaminase	1.00
		The present enzyme	1.18
	Sorghum	Streptomyces transglutaminase	1.00
		The present enzyme	1.02
Nuts	Chia seeds	Streptomyces transglutaminase	1.00
		The present enzyme	1.10

[0094]

Compared to samples untreated with enzymes, the use of the present enzyme resulted in the improvement of oil retentivity in lentils, corn, cashew nuts, almonds, walnuts, coconut, and industrial hemp. Among others, for lentils, corn, cashew nuts, almonds, and walnuts, the effect of improving oil retentivity by the present enzyme was higher than that by the use of Streptomyces-derived transglutaminase.

[0095]

(C: Emulsifiability evaluation)

A processed protein-containing dry composition was suspended in deionized water to prepare 30 mL of an aqueous suspension containing 1% by weight of protein. An emulsion composition was prepared by mixing 30 mL of the aqueous suspension with 10 mL of canola oil and then homogenizing the mixture at 10,000 rpm for 2 minutes. A 50-μL aliquot of the emulsion composition was taken immediately after the preparation, and was then added to 5 mL of a 0.1% SDS solution. The turbidity (A0) of the obtained solution was measured at an absorbance

wavelength of 500 nm, and "emulsifiability" was calculated using the equation below.

[Expression 2]

Emulsifiability (m^2/g) = (2 x 2.303 x A0) / (0.25 x Protein Mass)

[0096]

The emulsifiability of the enzyme-untreated protein-containing dry composition was also calculated in the same manner as described above. The relative value of the emulsifiability of the processed protein-containing dry composition, when the emulsifiability of the enzyme-untreated protein-containing dry composition was set at 1, was determined as an emulsifiability evaluation value. Additionally, for some protein materials, the relative value of the emulsifiability of the processed protein-containing dry composition, when the emulsifiability of the processed protein-containing dry composition treated with Streptomyces-derived transglutaminase was set at 1, was determined as an emulsifiability evaluation value.

[0097]

(Results)

The results are shown in the following tables.

[Table 10]

	Origin		Emulsifiability
Pulses	Peas	Untreated with enzymes	1.00
		The present enzyme	1.04
	Mung beans	Untreated with enzymes	1.00
		The present enzyme	1.02
	Fava beans	Untreated with enzymes	1.00
		The present enzyme	1.74
	Lentils	Untreated with enzymes	1.00
		The present enzyme	1.32
	Chickpeas	Untreated with enzymes	1.00
		The present enzyme	1.20
Grains	Rice	Untreated with enzymes	1.00
		The present enzyme	1.02
	Oats	Untreated with enzymes	1.00
		The present enzyme	1.14
Nuts	Chia seeds	Untreated with enzymes	1.00
		The present enzyme	1.20
Animal proteins	Milk casein	Untreated with enzymes	1.00
		The present enzyme	1.10
	Egg albumin	Untreated with enzymes	1.00
		The present enzyme	1.13

[Table 11]

	Origin		Emulsifiability
Pulses	Peas	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.97
		The present enzyme	1.04
	Mung beans	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.86
		The present enzyme	1.02
	Fava beans	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.68
		The present enzyme	1.74
	Lentils	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.27
		The present enzyme	1.32
	Chickpeas	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.96
		The present enzyme	1.20
Grains	Oats	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.05
		The present enzyme	1.14
Nuts	Chia seeds	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.01
		The present enzyme	1.20
Animal proteins	Milk casein	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.96
		The present enzyme	1.10
	Egg albumin	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.02
		The present enzyme	1.13

[Table 12]

	Origin		Emulsifiability
Animal proteins	Chicken powder	Streptomyces transglutaminase	1.00
		The present enzyme	1.02

[0098]

Compared to samples untreated with enzymes, the use of the present enzyme resulted in the improvement of emulsifiability in peas, mung beans, fava beans, lentils, chickpeas, oats, rice, chia seeds, milk casein, and egg albumin. Among others, for peas, mung beans, fava beans, lentils, chickpeas, oats, chia seeds, milk casein, and egg albumin, the effect of improving emulsifiability by the present enzyme was higher than that of Streptomyces-derived transglutaminase.

[0099]

(D: Emulsification stability evaluation)

Emulsification stability was evaluated using the emulsified composition prepared for evaluation of emulsifiability. A 50 μL aliquot of the emulsified composition was taken immediately after the preparation, and was then added to 5 mL of a 0.1% SDS solution, and its turbidity (A0) was measured at an absorbance wavelength of 500 nm. Another 50 μL aliquot of the emulsified composition was taken after standing for 10 minutes, and was then added to 5 mL of a 0.1% SDS solution. The turbidity (A10) of the obtained solution was measured at an absorbance wavelength of 500 nm. "Emulsification stability" was calculated using the equation below.

[Expression 3]

$$\text{Emulsification stability (\%)} = \{(A10 \times 10) / (A0 - A10)\} \times 100$$

[0100]

The emulsification stability of the enzyme-untreated protein-containing dry composition was also calculated in the same manner as described above. The relative value of the emulsification stability of the processed protein-containing dry composition, when the emulsification stability of the enzyme-untreated protein-containing dry composition was set at 1, was determined as an emulsification stability evaluation value. Additionally, for some protein materials, the relative

value of the emulsification stability of the processed protein-containing dry composition, when the emulsification stability of the processed protein-containing dry composition treated with Streptomyces-derived transglutaminase was set at 1, was determined as an emulsification stability evaluation value.

[0101]

(Results)

The results are shown in the following tables.

[Table 13]

	Origin		Emulsification stability
Pulses	Soybeans	Untreated with enzymes	1.00
		The present enzyme	3.07
	Mung beans	Untreated with enzymes	1.00
		The present enzyme	1.15
	Fava beans	Untreated with enzymes	1.00
		The present enzyme	1.24
	Lentils	Untreated with enzymes	1.00
		The present enzyme	1.62
	Chickpeas	Untreated with enzymes	1.00
		The present enzyme	1.04
Animal proteins	Milk casein	Untreated with enzymes	1.00
		The present enzyme	1.20
	Pork powder	Untreated with enzymes	1.00
		The present enzyme	1.27
	Chicken powder	Untreated with enzymes	1.00
		The present enzyme	1.23
	Egg albumin	Untreated with enzymes	1.00
		The present enzyme	1.17

[Table 14]

	Origin		Emulsification stability
Pulses	Soybeans	Untreated with enzymes	1.00
		Streptomyces transglutaminase	2.28
		The present enzyme	3.07
	Lentils	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.45
		The present enzyme	1.62
	Chickpeas	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.95
		The present enzyme	1.04
Animal proteins	Milk casein	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.08
		The present enzyme	1.20
	Pork powder	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.22
		The present enzyme	1.27
	Chicken powder	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.11
		The present enzyme	1.23

[Table 15]

	Origin		Emulsification stability
Pulses	Peas	Streptomyces transglutaminase	1.00
		The present enzyme	1.04
Grains	Rice	Streptomyces transglutaminase	1.00
		The present enzyme	1.07
	Oats	Streptomyces transglutaminase	1.00
		The present enzyme	1.01
Animal proteins	Beef powder	Streptomyces transglutaminase	1.00
		The present enzyme	1.04

[0102]

Compared to samples untreated with enzymes, the use of the present enzyme resulted in the improvement of emulsification stability in soybeans, mung beans, fava beans, lentils, chickpeas, milk casein, pork powder, chicken powder, and egg albumin. Among others, for soybeans, lentils, chickpeas, milk casein, pork powder, and chicken powder, the emulsification stability-improving effect by the present enzyme was higher than that by the use of Streptomyces-derived transglutaminase.

[0103]

(E: Foaming ability evaluation)

A processed protein-containing dry composition was suspended in deionized water to prepare 50 mL of a 0.5 wt% aqueous suspension. The aqueous suspension was homogenized at 18,000 rpm for 30 minutes, and was immediately transferred into a 100 mL graduated cylinder. The volume VF0 of the aqueous suspension, including foam, was measured, and the "foaming ability" was then calculated using the equation below.

[Expression 4]

Foaming ability (%) = 100 x (VF0 – 50) / 50

[0104]

The foaming ability of the enzyme-untreated protein-containing dry composition was also calculated in the same manner as described above. The relative value of the foaming ability of the processed protein-containing dry composition, when the foaming ability of the enzyme-untreated protein-containing dry composition was set at 1, was determined as a foaming ability evaluation value. Additionally, for some protein materials, the relative value of the foaming ability of the processed protein-containing dry composition, when the foaming ability of the processed protein-containing dry composition treated with Streptomyces-derived transglutaminase was set at 1, was determined as a foaming ability evaluation value.

[0105]

(Results)

The results are shown in the following tables.

[Table 16]

	Origin		Foaming ability
Pulses	Peas	Untreated with enzymes	1.00
		The present enzyme	1.75
	Fava beans	Untreated with enzymes	1.00
		The present enzyme	1.20

[Table 17]

	Origin		Foaming ability
Pulses	Peas	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.25
		The present enzyme	1.75

[Table 18]

	Origin		Foaming ability
Grains	Rice	Streptomyces transglutaminase	1.00
		The present enzyme	2.00

[0106]

Compared to samples untreated with enzymes, the use of the present enzyme resulted in the improvement of foaming ability in peas and fava beans. Among others, for peas, the foaming ability-improving effect by the present enzyme was higher than that by the use of Streptomyces-derived transglutaminase.

[0107]

(F: Foam stability evaluation)

A processed protein-containing dry composition was suspended in deionized water to prepare 50 mL of a 0.5 wt% aqueous suspension. The aqueous suspension was homogenized at 18,000 rpm for 30 minutes, and was immediately transferred into a 100 mL graduated cylinder, and thereafter, the volume VF0 of the aqueous suspension, including foam, was measured. Thirty minutes later, the volume VF30 of the aqueous suspension, including foam, was also measured, and the "foam stability" was calculated using the equation below.

[Expression 5]

Foam stability (%) = 100 x (VF30 / VF0)

[0108]

The foam stability of the enzyme-untreated protein-containing dry composition was also calculated in the same manner as described above. The relative value of the foam stability of the processed protein-containing dry composition, when the foam stability of the enzyme-untreated protein-containing dry composition was set at 1, was determined as a foam stability evaluation value. Additionally, for some protein materials, the relative value of the foam stability of the processed protein-containing dry composition, when the foam stability of the processed protein-containing dry composition treated with Streptomyces-derived transglutaminase was set at 1, was determined as a

foam stability evaluation value.

[0109]

(Results)

The results are shown in the following tables.

[Table 19]

	Origin		Foam stability
Animal proteins	Milk casein	Untreated with enzymes	1.00
		The present enzyme	1.01
	Egg albumin	Untreated with enzymes	1.00
		The present enzyme	1.05

[Table 20]

	Origin		Foam stability
Animal proteins	Egg albumin	Untreated with enzymes	1.00
		Streptomyces transglutaminase	1.00
		The present enzyme	1.05

[Table 21]

	Origin		Foam stability
Pulses	Peas	Streptomyces transglutaminase	1.00
		The present enzyme	1.01
	Mung beans	Streptomyces transglutaminase	1.00
		The present enzyme	1.04

[0110]

Compared to samples untreated with enzymes, the use of the present enzyme resulted in the

improvement of foam stability in milk casein and egg albumin. Among others, for egg albumin, the foam stability-improving effect by the present enzyme was higher than that by the use of Streptomyces-derived transglutaminase.

[0111]

(G: Protein solubility evaluation)

A processed protein-containing dry composition was suspended in deionized water to prepare a 5 wt% aqueous suspension. The prepared 5 wt% aqueous suspension was centrifuged at 15,000 x g for 5 minutes. The centrifuged supernatant was recovered, and the protein concentration (mg/mL) was then quantified using a Bradford reagent manufactured by Bio-Rad. The protein concentration (mg/mL) of the enzyme-untreated protein-containing dry composition was also quantified in the same manner as described above. The relative value of the protein concentration of the processed protein-containing dry composition, when the protein concentration of the enzyme-untreated protein-containing dry composition was set at 1, was determined as a protein solubility evaluation value. Additionally, for some protein materials, the relative value of the protein concentration of the processed protein-containing dry composition, when the protein concentration of the processed protein-containing dry composition treated with Streptomyces-derived transglutaminase was set at 1, was determined as a protein solubility evaluation value.

[0112]

(Results)

The results are shown in the following tables.

[Table 22]

	Origin		Protein solubility
Pulses	Mung beans	Untreated with enzymes	1.00
		Streptomyces transglutaminase	0.55
		The present enzyme	1.54

[Table 23]

	Origin		Protein solubility
Pulses	Lentils	Streptomyces transglutaminase	1.00
		The present enzyme	1.09
Animal proteins	Beef powder	Streptomyces transglutaminase	1.00
		The present enzyme	1.68

[0113]

Compared to samples untreated with enzymes, the use of the present enzyme resulted in the improvement of protein solubility in mung beans, and the protein solubility-improving effect by the present enzyme was higher than that by the use of Streptomyces-derived transglutaminase.

[0114]

Example 5: Confirmation of protein-modifying effects (production of food products and beverages with modified physical properties)

(1. Kamaboko (steamed fish paste))

Surimi (minced fish meat) was prepared by adding four times the amount of water to an all-purpose surimi powder (Suzuhiro Kamaboko Co., Ltd., protein content: 57.8 wt%). To 9 g of the surimi, 0.5 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL and 0.5 mL of water were added and mixed, and the obtained mixture was then quickly packed into a petri dish (56 mm in diameter, 16 mm in height) to form a molded shape. The petri dish was covered and was incubated at 50°C for 5 hours to obtain a kamaboko (treated with the present enzyme). The stress (maximum force recorded during the initial compression) of the obtained kamaboko was measured using a rheometer (manufactured by Sun Scientific Co., Ltd.). Separately, a kamaboko that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner as described above. The relative value of the stress of the kamaboko treated with the present enzyme, when the stress of an enzyme-untreated kamaboko was set at 1, was calculated as a stress improvement rate, and was

evaluated.

[0115]

(Results)

The results are shown in the following table.

[Table 24]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	3.63

[0116]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in a significant improvement in the stress of the kamaboko.

[0117]

(2. Yogurt)

One packet of starter culture (OHTA's ISAN Co., Ltd., "*Ousama no Yogurt Tanekin*") was added to 500 mL of milk (Meiji Co., Ltd., *Oishii Gyunyu* (delicious milk), protein content: 34 mg/mL). To 9 mL of this milk with the starter culture, 0.25 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL and 0.75 mL of water were added and mixed. After the mixing, the obtained mixture was poured into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and incubation was then carried out at 28°C for 24 hours to obtain yogurt (treated with the present enzyme). The stress of the obtained yogurt was measured using the same procedures as those described in "1. Kamaboko" above. Separately, yogurt that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. The relative value of the stress of the yogurt treated with the present enzyme, when the stress of an enzyme-untreated yogurt was set at 1, was calculated as a stress improvement rate, and was evaluated.

[0118]

(Results)

The results are shown in the following table.

[Table 25]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.93

[0119]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the yogurt.

[0120]

(3. Cheese)

To 30 g of commercially available cheddar cheese, 15 mL of 0.2 M phosphate buffer (pH 6.86) was added, and the cheese was completely dissolved in the buffer while stirring the mixture at 70°C. After the dissolution, the obtained mixture was cooled to 50°C, and was then dispensed in an amount of 20 g each into petri dishes (56 mm in diameter, 16 mm in height). Thereafter, 1 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added thereto in an amount of 1 mL each, and was then mixed. The petri dishes were covered, and incubation was then carried out at 50°C for 1 hour to obtain cheese samples (treated with the present enzyme, or treated with Streptomyces-derived transglutaminase). The stress of the obtained cheeses was measured using the same procedures as those described in "1. Kamaboko" above. Separately, cheese that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. Setting the stress of an enzyme-untreated cheese as 1, the relative value of the stress of the cheese treated with the present enzyme or the cheese treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0121]

(Results)

The results are shown in the following table.

[Table 26]

Conditions	Stress improvement rate
Untreated with enzymes	1.0
Streptomyces-derived transglutaminase	17.2
The present enzyme	58.4

[0122]

By using the present enzyme, a significantly higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0123]

(4. Hamburg steak)

Table salt was added to 200 g of ground beef (100% domestic beef, protein content: 17.1 wt%) to achieve a final concentration of 1%, and the obtained mixture was thoroughly blended until it became sticky. Subsequently, 140 mL of water was added to the mixture, and the mixture was blended again and was then divided into 20 g portions. To each portion of the meat mixture, 1 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL was added and mixed in, and the thus obtained mixture was shaped into a patty. The shaped patty was wrapped in a plastic wrap and was incubated at 50°C for 1 hour. After the incubation, both sides of the patty were baked in a frying pan over medium heat for 2 minutes per side. After the baking, it was cooled to room temperature to obtain a hamburg steak (treated with the present enzyme). The stress of the obtained hamburg steak was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a hamburg steak that did not undergo an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. The relative value of the stress of the hamburg steak treated with the present enzyme, when the stress of the enzyme-untreated hamburg steak was set at 1, was calculated as a stress improvement rate, and was evaluated.

[0124]

(Results)

The results are shown in the following table.

[Table 27]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.09

[0125]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the hamburg steak.

[0126]

(5. Chicken ham)

Chicken breast meat (100 g, protein content: 23.3 wt%) was softened by piercing it at regular intervals with a fork, and was then flattened using a rolling pin to achieve a uniform thickness. A pre-treatment was performed on the meat by applying 1.6 g of sugar to both sides, followed by rubbing 1.6 g of table salt into the meat. Inside a resealable plastic bag, 5 mL of water and 5 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL were each added to and mixed into 25 g of the pre-treated chicken breast meat. The bag was sealed after removing as much air as possible, and the contents were incubated at 50°C for 5 hours. After the incubation, the chicken breast meat was removed from the resealable plastic bag, was then rolled into a ham-like shape using aluminum foil, and was then molded. After the molding, the meat was boiled in boiling water. After the boiling, it was cooled to room temperature to obtain a chicken ham (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained chicken ham was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a chicken ham that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. Setting the stress of the enzyme-untreated chicken ham as 1, the relative stress values of the chicken ham treated with the present enzyme and the chicken ham treated with Streptomyces-derived transglutaminase were calculated as "stress improvement rates" and were evaluated.

[0127]

(Results)

The results are shown in the following table.

[Table 28]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.38
The present enzyme	1.80

[0128]

By using the present enzyme, a significantly higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0129]

(6. Chawanmushi (steamed egg custard))

Water (180 mL) was added to the beaten egg (protein content: 12.2 wt%) equivalent to one egg, and the mixture was strained through a tea strainer. To 9 mL of the beaten egg, 0.5 mL of water and 1 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL were added and mixed, and the obtained mixture was then incubated at 50°C for 4 hours. After the incubation, the reaction mixture was steamed in a boiling pot. Thereafter, the resultant was cooled to room temperature to obtain chawanmushi (treated with the present enzyme). Separately, chawanmushi that had not undergone an enzyme treatment (untreated with enzymes) was also prepared. Each of the resulting chawanmushi samples was scooped with a spoon, and the physical properties thereof were visually evaluated.

[0130]

(Results)

While the enzyme-untreated chawanmushi sample had a slightly grainy cross-section, the chawanmushi prepared using the present enzyme had a smooth cross-section.

[0131]

(7. Pancake)

100 g of "Cooking Flower" soft wheat flour (Nisshin Seifun Welna Inc., protein content: 8 wt%) and 4 g of "Super Camellia" dry yeast for home bread makers (Nisshin Seifun

Welna Inc.) were thoroughly mixed with 100 mL of water, and appropriate amounts of sugar and beaten egg were added to the mixture. The obtained mixture was divided into 20 g portions, and 1 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL was added to and mixed with each portion, followed by incubation at 50°C for 1 hour. After the incubation, both sides of the mixture were baked in a frying pan over medium heat for 2 minutes per side. After the baking, it was cooled to room temperature to obtain a pancake (treated with the present enzyme). The stress of the obtained pancake was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a pancake that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. The relative value of the stress of the pancake treated with the present enzyme, when the stress of the enzyme-untreated pancake was set at 1, was calculated as a stress improvement rate, and was evaluated.

[0132]

(Results)

The results are shown in the following table.

[Table 29]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.32

[0133]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the pancake.

[0134]

(8. Tofu (soybean curd))

To a mixture of 9 mL of soy milk (Sujahta Meiraku Group, *Tofu mo dekimasu Yuki Tonyu* (Organic soy milk suitable for tofu making), protein content: 50 mg/mL) and 0.5 mL of nigari solution (magnesium chloride), 0.5 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL was added and mixed. After the mixing, the mixture was poured into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and

incubation was carried out at 40°C for 20 hours to obtain tofu (treated with the present enzyme). The stress of the obtained tofu was measured using the same procedures as those described in "1. Kamaboko" above. Separately, tofu that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. Setting the stress of the enzyme-untreated tofu as 1, the relative stress value of the tofu treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0135]

(Results)

The results are shown in the following table.

[Table 30]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.70

[0136]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the tofu.

[0137]

(9. Soy milk yogurt)

One packet of starter culture (OHTA's ISAN Co., Ltd., "*Ousama no Yogurt Tanekin*") was added to 500 mL of soy milk (Sujahta Meiraku Group, *Tofu mo dekimasu Yuki Tonyu* (Organic soy milk suitable for tofu making), protein content: 50 mg/mL). To 9 mL of the soy milk containing the starter culture, 0.5 mL of water and 0.5 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL were added and mixed. After the mixing, the mixture was poured into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and incubation was carried out at 28°C for 24 hours to obtain soy milk yogurt (treated with the present enzyme). The stress of the obtained soy milk yogurt was measured using the same procedures as those described in "1. Kamaboko" above. Separately, soy milk yogurt that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. Setting the stress of the enzyme-untreated soy

milk yogurt as 1, the relative value of the stress of the soy milk yogurt treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0138]

(Results)

The results are shown in the following table.

[Table 31]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.93

[0139]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the soy milk yogurt.

[0140]

(10. Alternative hamburg steak)

A granular vegetable protein (Marukome Co., Ltd., "*Daizu Labo Daizu no Oniku Minchi* (soy meat mince)" dry type, protein content: 45.4 wt%) was rehydrated and swollen in six times its weight of hot water for 10 minutes. To 25 g of the swollen granular vegetable protein, 1 g of methylcellulose (Shin-Etsu Chemical Co., Ltd., "Metolose") and 2.75 g of pea protein (Roquette, "Nutralys F85M") were added, finally, 20 mL of water was added and mixed. To the resulting raw patty obtained by mixing, 1 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added and mixed. The mixture was incubated at room temperature for 1 hour. After the incubation, both sides of the patty were baked in an oven at 150°C, grilling each side for 5 minutes. After the baking, the resulting patty was cooled to room temperature to obtain an alternative hamburg steak (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained alternative hamburg steak was measured using the same procedures as those described in "1. Kamaboko" above. Separately, an alternative hamburg steak that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. Setting the stress of the enzyme-untreated alternative hamburg steak as 1,

the relative stress value of the alternative hamburg steak treated with the present enzyme or with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0141]

(Results)

The results are shown in the following table.

[Table 32]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.00
The present enzyme	1.10

[0142]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0143]

(11. Alternative cheese)

As materials for preparing an alternative cheese, 18.8 g of pea protein (Roquette, LYSAMINE GPS), 10 g of tapioca starch, 2 g of canola oil, 12 g of coconut oil (pre-melted), 0.6 g of table salt, 1.8 g of calcium phosphate, and 1 g of carrageenan were blended, and thereafter; water was added to the mixture to a total weight to 100 g, and the obtained mixture was then blended. The mixture was homogenized at 1,000 rpm for 10 minutes, while it was kept warm at 50°C. After the homogenization, 10 g aliquots of the mixture were dispensed into petri dishes (56 mm diameter, 16 mm height), and 0.5 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added to each aliquot and mixed therewith. The dishes were covered, and incubation was carried out at 40°C for 20 hours to obtain an alternative cheese (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained alternative cheese was measured using the same procedures as those described in "1. Kamaboko" above. Separately, an alternative cheese that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was

also measured in the same manner described above. Setting the stress of the enzyme-untreated alternative cheese as 1, the relative stress value of the alternative cheese treated with the present enzyme or the alternative cheese treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0144]

(Results)

The results are shown in the following table.

[Table 33]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	2.35
The present enzyme	6.65

[0145]

By using the present enzyme, a significantly higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0146]

(12. Bovine bone gelatin, porcine skin gelatin, and fish gelatin)

Gelatin solutions were prepared by dissolving each gelatin shown in the table below in a 50 mM phosphate buffer to achieve a final concentration of 5% by mass.

[Table 34]

Product name	Protein content	Manufacturer
Bovine bone gelatin	100%	Nitta Gelatin Inc.
Porcine skin gelatin	100%	Nitta Gelatin Inc.
Fish gelatin	100%	Nitta Gelatin Inc.

[0147]

To 9 mL of each gelatin solution, 0.5 mL of water and 0.5 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL were added and mixed. After the mixing, the mixture was poured into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and incubation was then carried out at 40°C for 20 hours to obtain a coagulated

gelatin (treated with the present enzyme). The stress of the obtained coagulated gelatin was measured using the same procedures as those described in "1. Kamaboko" above. It is to be noted that the gelatin did not coagulate when the enzyme treatment was not performed.

[0148]

(Results)

The results are shown in the following table.

[Table 35]

Material	Bovine bone gelatin	Porcine skin gelatin	Fish gelatin
	Stress (N)	Stress (N)	Stress (N)
Untreated with enzymes	0	0	0
The present enzyme	2.9	7.3	0.7

[0149]

Coagulation was observed in all types of gelatin, when the present enzyme was used.

[0150]

(13. Milk gel/soy milk gel)

To 10 mL of milk (Meiji Co., Ltd., *Oishii Gyunyu* (delicious milk)) or 10 mL of soy milk (Sujahta Meiraku Group, *Tofu mo dekimasu Yuki Tonyu* (Organic soy milk suitable for tofu making)), a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added and mixed. After the mixing, the mixture was poured into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and incubation was then carried out at 40°C for 20 hours to obtain milk gel or soy milk gel (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained gel was measured using the same procedures as those described in "1. Kamaboko" above. It is to be noted that neither the milk gel nor the soy milk gel coagulated when the enzyme treatment was not performed.

[0151]

(Results)

The results are shown in the following table.

[Table 36]

Conditions	Milk gel	Soy milk gel
	Stress (N)	Stress (N)
Untreated with enzymes	0	0
Streptomyces-derived transglutaminase	0.2	0.2
The present enzyme	0.5	0.7

[0152]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0153]

(14. Egg white gel)

Egg white (protein content: 10.1 wt%) was separated from chicken eggs, 180 mL of water was then added to the egg white, and the mixture was filtered through a tea strainer. To 9 mL of the egg white solution, 0.5 mL of water and 1 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL were added and mixed. After the mixing, the obtained mixture was poured into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and incubation was then carried out at 50°C for 4 hours. After the incubation, the obtained mixture was steamed in a boiling pot. The resultant was cooled to room temperature to obtain an egg white gel (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained egg white gels were measured using the same procedures as those described in "1. Kamaboko" above. Separately, an egg white gel that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. Setting the stress of the enzyme-untreated egg white gel as 1, the relative stress value of the egg white gel treated with the present enzyme or the egg white gel treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and were evaluated.

[0154]

(Results)

The results are shown in the following table.

[Table 37]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.53
The present enzyme	1.68

[0155]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0156]

(15. Chicken egg yolk gel)

To 9 mL of egg yolk separated from a chicken egg (protein content: 16.5 wt%), 0.5 mL of water and 0.5 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL were added and mixed. After the mixing, the obtained mixture was poured into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and incubation was then carried out at 50°C for 5 hours to obtain a chicken egg yolk gel (treated with the present enzyme). The stress of the obtained chicken egg yolk gel were measured using the same procedures as those described in "1. Kamaboko" above. Separately, a chicken egg yolk gel that had not undergone an enzyme treatment (untreated with enzymes) was prepared, and the stress thereof was also measured in the same manner described above. Setting the stress of the enzyme-untreated chicken egg yolk gel as 1, the relative value of the stress of the chicken egg yolk gel treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0157]

(Results)

The results are shown in the following table.

[Table 38]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.85

[0158]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the chicken egg yolk gel.

[0159]

(16. Thermoreversible casein gel)

Milk casein was suspended in a 50 mM phosphate buffer to a final concentration of 11.1% by mass, and the pH of the solution was adjusted to pH 7 using sodium hydroxide. Subsequently, the milk casein suspension was heated in boiling water until it was completely dissolved. To 9 mL of the casein solution, 0.75 mL of water and 0.25 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL were added and mixed. After thorough mixing, the obtained mixture was incubated at 40°C for 20 hours to induce gelation. The resulting gelled product was subjected to an autoclave treatment (under conditions at 120°C for 20 minutes), and was then cooled to room temperature.

[0160]

(Results)

The gel formed by Streptomyces-derived transglutaminase retained its gel shape, even after autoclaving. In contrast, the gel formed by the present enzyme turned into a liquid after autoclaving, but it re-gelled upon cooling to room temperature. This thermoreversible gel formation occurred even when the heat treatment (autoclave treatment) and cooling cycle was repeated, at least, three times.

[0161]

(17. Protein cross-linking of oat milk)

When the present enzyme was allowed to act on oat milk, cross-linking of oat proteins could be confirmed.

[0162]

(18. Tuna nuggets)

To 70 g of tuna flakes containing a seasoning liquid (Inaba Light Tuna Flakes, Inaba Foods Co., Ltd., protein content: 11.8 wt%), 37.5 g of wheat flour and a small amount of table salt were added and mixed. The resulting fish meat mixture was divided into 20 g portions, and 1 mL of a

solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was then added to each portion, followed by further mixing. The obtained mixture was packed into a petri dish (56 mm diameter, 16 mm height) and was molded, the petri dish was then covered, and incubation was then carried out at 50°C for 2 hours. After the incubation, the obtained mixture was removed from the petri dish, and both sides of the mixture was pan-fried in salad oil heated to 170°C, cooking for 2 minutes on each side. Excess oil was drained using a wire rack, and the product was then cooled to room temperature to obtain tuna nuggets (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained tuna nuggets was measured using the same procedures as those described in "1. Kamaboko" above. Separately, tuna nuggets (untreated with enzymes) were prepared and their stress was measured by performing the same procedures as described above with the exception that no enzyme treatment was conducted. Setting the stress of the enzyme-untreated tuna nuggets as 1, the relative stress value of the tuna nuggets treated with the present enzyme or with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0163]

(Results)

The results are shown in the following table.

[Table 39]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.25
The present enzyme	1.32

[0164]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0165]

(19. Butter)

To 15 g of salted butter (*Yukijirushi Hokkaido Butter*, Megmilk Snow Brand Co., Ltd.,

protein content: 0.6 wt%), 2.25 mL of a 0.2 M phosphate buffer (pH 6.86) was added, and the mixture was then stirred at 50°C for 15 minutes. Thereafter, 0.75 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added to the reaction mixture obtained above, and the obtained mixture was further blended. After the mixing, the thus obtained mixture was incubated at 50°C for 1 hour. After the incubation, the butter was poured into a petri dish (56 mm in diameter, 16 mm in height), and the petri dish was covered, and was then cooled in a refrigerator overnight. After the cooling overnight, the resultant was left to reach room temperature, so as to obtain a butter (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained butter was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a butter (untreated with enzymes) was prepared by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated butter as 1, the relative stress value of the butter treated with the present enzyme or the butter treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0166]

(Results)

The results are shown in the following table.

[Table 40]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.17
The present enzyme	1.77

[0167]

By using the present enzyme, a significantly higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0168]

(20. Tonkatsu (Japanese pork cutlet))

A 35 g piece of pork round (protein content: 20.5 wt%) was softened by piercing it with a

fork at regular intervals, was then wrapped in a plastic wrap, and was then flattened with a rolling pin to achieve a uniform thickness. A small amount of table salt was rubbed onto both sides of the thickness-uniform pork to blend the salt in it. The pork was placed in a resealable plastic bag, and 5 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL and 5 mL of water were added to the pork to further blend them in it. The bag was sealed after removing as much air as possible, and the contents were incubated at 40°C for 3 hours. After the incubation, the pork was removed from the resealable plastic bag, and the entire meat was lightly dusted with wheat flour. The resulting meat was further dipped in beaten egg and was then coated with breadcrumbs. The meat was deep-fried in salad oil heated to 180°C for 2 minutes and 30 seconds, and excess oil was then drained off using a wire rack. The resultant was allowed to cool to room temperature, so as to obtain a tonkatsu (treated with the present enzyme). The stress of the obtained tonkatsu was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a tonkatsu (untreated with enzymes) was prepared by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated tonkatsu as 1, the relative value of the stress of the tonkatsu treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0169]

(Results)

The results are shown in the following table.

[Table 41]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.89

[0170]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in a significant improvement in the stress of the tonkatsu.

[0171]

(21. Sausage)

To 50 g of coarsely ground pork (protein content: 17.7 wt%), 1.25 g of table salt, 1.875 g of sugar, 1.25 mL of water, and 5 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL were added and mixed. Separately, a 20 cm-long sheep casing that had been soaked in water for 15 minutes was prepared. The seasoned meat mixture was stuffed into the sheep casing using a sausage stuffer, was then wrapped in a plastic wrap, and was then incubated at 50°C for 3.5 hours. Each sausage contained 50 g of the meat mixture and had a total length of 6 cm. After the incubation, the sausage was boiled in water at approximately 80°C for 20 minutes, and thereafter, the sausage as a whole was baked in a frying pan over medium heat for 5 minutes. After the baking, it was cooled to room temperature to obtain a sausage (treated with the present enzyme). The stress of the obtained sausage was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a sausage (untreated with enzymes) was prepared by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated sausage as 1, the relative value of the stress of the sausage treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0172]

(Results)

The results are shown in the following table.

[Table 42]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.64

[0173]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the sausage.

[0174]

(22. Chicken meatballs)

An appropriate amount of chicken thigh meat (protein content: 16.6 wt%) was processed in

a food processor to create minced meat. To 300 g of the resulting minced chicken thigh meat, 1.25 g of table salt and 5 g of sugar were added, and the mixture was then kneaded thoroughly until it became sticky. Subsequently, beaten egg (equivalent to one chicken egg) and 15 g of wheat flour were further added thereto, and the meat mixture was blended until they were well blended in the meat. The meat mixture was divided into 25 g portions, and to each portion, 1 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL was added and mixed. The thus obtained mixture was packed into a petri dish (56 mm in diameter, 16 mm in height) and was then molded, and the petri dish was then covered, followed by incubation at 50°C for 1 hour. After the incubation, the mixture was boiled in boiling water for 5 minutes. After the boiling, the resultant was cooled to room temperature to obtain chicken meatballs (treated with the present enzyme). The stress of the obtained chicken meatballs were measured using the same procedures as those described in "1. Kamaboko" above. Separately, chicken meatballs (untreated with enzymes) were obtained by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated chicken meatballs as 1, the relative value of the stress of the chicken meatballs treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0175]

(Results)

The results are shown in the following table.

[Table 43]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.18

[0176]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the chicken meatballs.

[0177]

(23. Beef steak)

A 40 g piece of beef tenderloin (protein content: 20.5 wt%) was pierced with a fork at regular intervals, was then wrapped in a plastic wrap, and was flattened to a uniform thickness using a rolling pin. A small amount of table salt was rubbed onto both sides of the thickness-uniform beef tenderloin. The beef tenderloin was placed in a resealable plastic bag, and 5 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL and 5 mL of water were added to the beef tenderloin to further blend them in it. The bag was sealed after removing as much air as possible, and the contents were incubated at 40°C for 4 hours. After the incubation, the beef tenderloin was removed from the resealable plastic bag, and both sides of the beef tenderloin were baked in a frying pan over medium heat for 2 minutes per side. After the baking, the resultant was cooled to room temperature to obtain the beef steak (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained beef steak was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a beef steak (untreated with enzymes) was prepared by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated beef steak set as 1, the relative stress value of the beef steak treated with the present enzyme or the beef steak treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0178]

(Results)

The results are shown in the following table.

[Table 44]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.33
The present enzyme	1.65

[0179]

By using the present enzyme, a higher stress could be obtained than the case of using

Streptomyces-derived transglutaminase.

[0180]

(24. Cookies)

50 g of strong wheat flour (Nisshin Camellia Strong Wheat Flour (Nisshin Seifun Welna Inc.), protein content: 12.6 wt%), 25 g of salted butter (*Yukijirushi Hokkaido Butter* (Megmilk Snow Brand Co., Ltd.)), 25 g of sugar, 30 g of beaten egg, 0.4 g of baking powder, and a small amount of table salt were added to a bowl, and were thoroughly mixed. The cookie dough obtained by the mixing was divided into 15 g portions, and 1 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added to and mixed with each portion. The bowl containing the cookie dough was covered with a plastic wrap, and incubation was then carried out at 50°C for 4 hours. Thereafter, the dough was allowed to rest in a refrigerator for 1 hour and was then molded using a rolling pin. The dough was baked in an oven preheated to 160°C, with the top and bottom sides baked for 10 minutes each (baking temperature: 160°C). After the baking, the cookies were cooled to room temperature to obtain cookies (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained cookies was measured using the same procedures as those described in "1. Kamaboko" above. It is to be noted that the measurement was terminated if a cookie cracked during the measurement. Separately, cookies (untreated with enzymes) were obtained by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated cookies as 1, the relative value of stress of the cookies treated with the present enzyme or the cookies treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0181]

(Results)

The results are shown in the following table.

[Table 45]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.02
The present enzyme	1.13

[0182]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0183]

(25. Tuna fillet)

A 20 g raw tuna fillet (protein content: 25.4 wt%) and 2 mL of water were placed in a resealable plastic bag and were well mixed. To this mixture, 2 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added, and they were further mixed. The bag was sealed after removing as much air as possible, and incubation was then carried out at 40°C for 4 hours. After the incubation, the tuna fillet was removed from the resealable plastic bag, and was then rinsed under running water. Excess water was drained, and the fillet was cooled to room temperature to obtain an enzyme-treated tuna fillet (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained tuna fillet was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a tuna fillet (untreated with enzymes) was obtained by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated tuna fillet as 1, the relative stress value of the tuna fillet treated with the present enzyme or the tuna fillet treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0184]

(Results)

The results are shown in the following table.

[Table 46]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.58
The present enzyme	1.81

[0185]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0186]

(26. Squid fillets)

A 20 g portion of raw squid fillets (protein content: 18.6 wt%) and 2 mL of water were placed in a resealable plastic bag, and were well mixed. To this mixture, 2 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL was added, and they were further mixed. The bag was sealed after removing as much air as possible, and incubation was carried out at 40°C for 4 hours. After the incubation, the squid fillets were removed from the resealable plastic bag, and were then rinsed under running water. Excess water was drained, and the fillets were cooled to room temperature to obtain enzyme-treated squid fillets (treated with the present enzyme). The stress of the obtained squid fillets was measured using the same procedures as those described in "1. Kamaboko" above. Separately, squid fillets (untreated with enzymes) were obtained by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated squid fillets as 1, the relative value of the stress of the squid fillets treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0187]

(Results)

The results are shown in the following table.

[Table 47]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	3.38

[0188]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the squid fillets.

[0189]

(27. Scallop adductor muscle)

A 160 g raw scallop adductor muscle (protein content: 16.9 wt%) and 2 mL of water were placed in a resealable plastic bag, and were well mixed. To this mixture, 2 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL was added, and they were further mixed. The bag was sealed after removing as much air as possible, and incubation was carried out at 40°C for 4 hours. After the incubation, the scallop adductor muscle was removed from the resealable plastic bag, and were then rinsed under running water. Excess water was drained, and the scallop adductor muscle was cooled to room temperature to obtain an enzyme-treated scallop adductor muscle (treated with the present enzyme). The stress of the obtained scallop adductor muscle was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a scallop adductor muscle (untreated with enzymes) were obtained by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated scallop adductor muscle as 1, the relative value of the stress of the scallop adductor muscle treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0190]

(Results)

The results are shown in the following table.

[Table 48]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.28

[0191]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the scallop adductor muscle.

[0192]

(28. Tarako (cod roe))

A thin membrane was removed from tarako (cod roe) (protein content: 24.0 wt%), and a 5 g portion was weighed and was then placed in a beaker. To this portion, 0.5 mL of water and 0.5 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL were added and mixed. The aforementioned tarako mixture was packed into a petri dish (56 mm in diameter, 16 mm in height), the petri dish was then covered, and incubation was then carried out at 40°C for 4 hours. After the incubation, the mixture was cooled to room temperature to obtain an enzyme-treated tarako (treated with the present enzyme or treated with Streptomyces-derived transglutaminase). The stress of the obtained tarako was measured using the same procedures as those described in "1. Kamaboko" above. Separately, a tarako sample (untreated with enzymes) was prepared by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated tarako as 1, the relative stress value of the stress of the tarako treated with the present enzyme or the tarako treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0193]

(Results)

The results are shown in the following table.

[Table 49]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	2.40
The present enzyme	3.70

[0194]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0195]

(29. Ice cream)

Into a pot, 250 mL of milk (Meiji Co., Ltd., *Oishii Gyunyu* (delicious milk), protein content: 34 mg/mL), 50 g of sugar, and 30 g of beaten egg (protein content: 12.2 wt%) were added, and they were mixed well, and the obtained mixture was heated over low heat until it was thickened to prepare a milk mixture. After the mixture had been cooled slightly, 3 mL of a solution containing either the enzyme prepared in Example 1 or Streptomyces-derived transglutaminase at a concentration of 1.0 mg/mL was added and mixed into 60 g of the milk mixture, incubation was then carried out at 40°C for 3 hours. After the incubation, the enzyme-treated milk mixture was divided into 20 g portions in petri dishes, and the petri dishes were then covered, followed by freezing them overnight in a freezer at -25°C. After the overnight freezing, enzyme-treated ice cream samples (treated with the present enzyme or treated with Streptomyces-derived transglutaminase) were obtained. The stress of the obtained ice cream samples was measured using the same procedures as those described in "1. Kamaboko" above. Separately, an ice cream sample (untreated with enzymes) was prepared by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated ice cream as 1, the relative value of the stress of the ice cream treated with the present enzyme or the ice cream treated with Streptomyces-derived transglutaminase was calculated as a stress improvement rate, and was evaluated.

[0196]

(Results)

The results are shown in the following table.

[Table 50]

Conditions	Stress improvement rate
Untreated with enzymes	1.00
Streptomyces-derived transglutaminase	1.45
The present enzyme	3.50

[0197]

By using the present enzyme, a higher stress could be obtained than the case of using Streptomyces-derived transglutaminase.

[0198]

(30. White rice/brown rice)

To 8.65 g of rinsed white rice (protein content: 6.1 wt%) or brown rice (protein content: 6.8 wt%), 8 mL of water and 2 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL were added and mixed. The obtained mixture was incubated at 40°C for 4 hours, and was then cooked. After cooling the rice slightly, it was molded to obtain enzyme-treated white rice or brown rice (treated with the present enzyme). The stress of the obtained white rice or brown rice was measured using the same procedures as those described in "1. Kamaboko" above. Separately, white rice or brown rice samples (untreated with enzymes) were obtained by performing the same procedures as described above with the exception that no enzyme treatment was conducted, and the stress thereof was then measured. Setting the stress of the enzyme-untreated white rice or brown rice set as 1, the relative value of the stress of the white rice or brown rice treated with the present enzyme was calculated as a stress improvement rate, and was evaluated.

[0199]

(Results)

The results are shown in the following table.

[Table 51]

White rice

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.16

[Table 52]

Brown rice

Conditions	Stress improvement rate
Untreated with enzymes	1.00
The present enzyme	1.26

[0200]

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in an improvement in the stress of the white rice/brown rice.

[0201]

(31. Pasta)

115 g of Durum wheat semolina (protein content: 13.3 wt%), 50 g of beaten egg (protein content: 12.2 wt%), and 5 g of table salt were mixed and kneaded for 10 minutes until the dough came together. To the aforementioned dough, 10 mL of a solution containing the enzyme prepared in Example 1 at a concentration of 1.0 mg/mL was added, and the mixture was further kneaded for an additional 10 minutes. The dough was wrapped in a plastic wrap and was then allowed to rest in a refrigerator for 30 minutes. Thereafter, the dough was kneaded again until it became uniform, and was then rolled out with a rolling pin to a thickness of 1 mm. The rolled-out dough was cut into 5 cm x 5 cm squares. The cut dough pieces were arranged on a tray, were then covered with a plastic wrap, and were then incubated at 40°C for 2 hours. After the incubation, the dough was boiled in boiling water for 2 minutes, and was then rinsed in cold water. The dough was cooled to room temperature to obtain an enzyme-treated pasta (treated with the present enzyme). Separately, a pasta (untreated with enzymes) was obtained by performing the same procedures as described above with the exception that no enzyme treatment was conducted. Two sensory evaluators

evaluated the tactile feel of the boiled dough for both the enzyme-untreated pasta and the pasta treated with the present enzyme.

[0202]

(Results)

Compared to the sample untreated with enzymes, the use of the present enzyme resulted in a dough with a chewy texture after boiling.

[0203]

Example 6: Preparation of enzyme having protein deamidation activity and transglutaminase activity derived from *Crossiella cryophila* (the present enzyme 2) and enzyme having protein deamidation activity and transglutaminase activity derived from *Krasilnikovia cinnamomea* (the present enzyme 3)

1. Production of transformants

Using an expression vector for the microorganisms of Streptomyces sp., a sequence of interest was incorporated into the expression vector. PCR amplification was performed, and transformants (a heterologous expression strain derived from *C. cryophila* and a heterologous expression strain derived from *K. cinnamomea*) were obtained using ordinary methods. Sequence analysis was performed on the obtained transformants to confirm that the sequences of interest had been incorporated therein.

[0204]

2. *C. cryophila*-derived heterologous expression strain, and *K. cinnamomea*-derived heterologous expression strain

The obtained transformants were each cultured in Trypto-Sawyer Broth Medium (manufactured by Nissui Pharmaceutical Co., Ltd.) at 32°C for 4 days, and the supernatant was then recovered. The obtained supernatant was dialyzed with 20 mM phosphate buffer (pH 6) according to an ordinary method. The dialyzed sample was subjected to ion exchange chromatography using a cation exchange column (SP Sepharose FF, manufactured by GE HealthCare) equilibrated with the same buffer as described above. Thereafter, the resulting sample was further purified using His tag protein purification column (His trap HP, manufactured by Cytiva) to obtain each enzyme sample.

[0205]

The amino acid sequences and nucleotide sequences of an enzyme having a protein deamidation activity and a transglutaminase activity, derived from *C. cryophila*, are shown below.

Amino acid sequence (only mature form sequence) (SEQ ID NO: 6)

GLPIAPPLRPGVESKTWKVADYIKAWETYHGRPMTADERENLARGCIGVTVVNLERGDV
GNPPLGLSFGTFGKAREVQAALNEIIKSKPSAAQYAAAVRSHPLLSKLENVQRALPADLN
TGELTAAIFSKRFYSKQNPNWTDEFAEKMYRANARGQVDMNSYRYVARPGYVNFDYG
WYDENTRNWWHANHAEPGMKVYQSTFDYYSRDLLDFDRQVFTVAFAKKV

[0206]

Amino acid sequence (full length) * The underlined part indicates the mature body sequence. (SEQ ID NO: 7)

MRNSVLIRTLTTAALGLGLLIPGAAAATAAPAPSTAAAVSIV<u>GLPIAPPLRPGVESKTWKV
ADYIKAWETYHGRPMTADERENLARGCIGVTVVNLERGDVGNPPLGLSFGTFGKAREV
QAALNEIIKSKPSAAQYAAAVRSHPLLSKLENVQRALPADLNTGELTAAIFSKRFYSKQNP
NWTDEFAEKMYRANARGQVDMNSYRYVARPGYVNFDYGWYDENTRNWWHANHAEP
GMKVYQSTFDYYSRDLLDFDRQVFTVAFAKKV</u>

[0207]

Nucleotide sequence (mature body) (SEQ ID NO: 8)

ggcctccccatcgccccccgctgcgcccgggcgtcgagtcgaagacgtggaaggtcgcggactacatcaaggcgtgggagacgtaccatg
gccgcccgatgaccgcggacgaacgggagaacctcgcgcggggctgcatcggggtcaccgtggtcaacctcgagcggggcgacgtgggg
aatccgccgctcggcctctcgttcggcacgttcggcaaagcgcgggaggtccaagcggcgctcaacgagatcatcaagtcgaagccgtcggc
ggcgcagtacgcggccgcggtccgctcgcacccccctgctctcgaagctcgagaacgtgcagcgggccctcccggcggatctcaacacgggc
gagctcacggcggcgatcttctcgaagcggttctactcgaagcagaacccgaactggacggacgagttcgcggagaagatgtaccgggcgaa
cgcgcggggccaagtcgacatgaactcgtaccggtacgtcgcgcggccgggctacgtcaacttcgactacggctggtacgacgagaacacg
cggaactggtggcacgcgaatcacgcggagccgggcatgaaggtctatcagtcgacgttcgactactactcgcgggatctcctcgacttcgacc
ggcaagtcttcacggtcgcgttcgcgaagaaggtc

[0208]

Nucleotide sequence (full length) (SEQ ID NO: 9)

atgcggaactccgtgctcatccgcacgctgaccaccgccgccctcggcctgggcctcctgatccccggggcggcggccgcgacggcggccc
cggcgccctccaccgcggcggccgtctccattgtcggcctccccatcgccccccgctgcgcccgggcgtcgagtcgaagacgtggaaggtc

gcggactacatcaaggcgtgggagacgtaccatggccgcccgatgaccgcggacgaacgggagaacctcgcgcggggctgcatcggggtc
accgtggtcaacctcgagcggggcgacgtggggaatccgccgctcggcctctcgttcggcacgttcggcaaagcgcgggaggtccaagcgg
cgctcaacgagatcatcaagtcgaagccgtcggcggcgcagtacgcggccgcggtccgctcgcacccccctgctctcgaagctcgagaacgtg
cagcgggccctcccggcggatctcaacacgggcgagctcacggcggcgatcttctcgaagcggttctactcgaagcagaacccgaactggac
ggacgagttcgcggagaagatgtaccgggcgaacgcgcggggccaagtcgacatgaactcgtaccggtacgtcgcgcggccgggctacgt
caacttcgactacggctggtacgacgagaacacgcggaactggtggcacgcgaatcacgcggagccgggcatgaaggtctatcagtcgacgt
tcgactactactcgcgggatctcctcgacttcgaccggcaagtcttcacggtcgcgttcgcgaagaaggtc

[0209]

The amino acid sequences and nucleotide sequences of an enzyme having a protein deamidation activity and a transglutaminase activity, derived from *K. cinnamomea*, are shown below.

Amino acid sequence (only mature form sequence) (SEQ ID NO: 10)

TESDPRSPTPARTATQAPIAEVRPAERSLANANAATATELTPLAPALPPGVSTRTWSVEDF
VELFERKYGRPMTQDERNALARGCIGVTTVNLERGNINPPLGMSFGTFATSRDVQNAIND
ILATNPSRTQFVAAVAQHPLLSRIDNVTDSLPGGPTSQWTAVIFSKRFYSKQDPSWTDEQA
DQAFRPDPATGQVDMTDYRYRAKPGYVNFDYGWLDEGSGNWWHANHAEPGMKVYQ
STLRHYSRPLLDFDRQVFSVTFGRVHP

[0210]

Amino acid sequence (full length) * The underlined part indicates the mature body sequence. (SEQ ID NO: 11)

MPKRLLRLFVIFVAGLSISTMVAAPALA<u>TESDPRSPTPARTATQAPIAEVRPAERSLANAN
AATATELTPLAPALPPGVSTRTWSVEDFVELFERKYGRPMTQDERNALARGCIGVTTVNL
ERGNINPPLGMSFGTFATSRDVQNAINDILATNPSRTQFVAAVAQHPLLSRIDNVTDSLPG
GPTSQWTAVIFSKRFYSKQDPSWTDEQADQAFRPDPATGQVDMTDYRYRAKPGYVNFD
YGWLDEGSGNWWHANHAEPGMKVYQSTLRHYSRPLLDFDRQVFSVTFGRVHP</u>

[0211]

Nucleotide sequence (mature body) (SEQ ID NO: 12)

accgaatccgacccgcggtcccccaccccgcgcgcacggccacgcaagcgccgatcgcggaagtgcggccggcggaacgctcgctggc
gaacgccaatgccgccaccgccaccgaactgacccccctcgcccccgccctccccccgggggtgtcgacgcggacgtggtcggtcgagga

cttcgtcgagctcttcgagcggaagtacgggcggccgatgacccaagacgaacgcaatgccctcgcgcggggctgcatcggcgtcacgacg
gtcaacctcgagcggggcaacatcaacccgccgctcggcatgtcgttcggcacgttcgcgacgtcgcgggacgtgcagaacgcgatcaacga
catcctcgcgaccaatccctcccggacccaatttgtggcggccgtggcgcagcacccgctcctctcgcggatcgacaacgtcacggactcgctc
ccgggcggcccgacgtcgcagtggacggcggtcatcttctcgaagcggttctactcgaagcaagacccgtcgtggacggacgagcaagcgg
atcaagcgttccgccccgatccggcgacgggccaagtcgacatgacggactaccggtaccgggcgaagccgggctacgtcaacttcgactac
ggctggctcgacgagggctcgggcaactggtggcacgcgaaccacgcggagccggggatgaaggtgtatcagtcgacgctccggcactact
cgcggccgctcctcgacttcgaccggcaagtcttctcggtcacgttcggccgggtccacccg

[0212]

Nucleotide sequence (full length) (SEQ ID NO: 13)

atgccgaagcggctcctccggctcttcgtcatcttcgtcgcgggcctctcgatttcgaccatggtggccgccccggccctcgcgaccgaatccga
cccgcggtcccccaccccgcgcgcacggccacgcaagcgccgatcgcggaagtgcggccggcggaacgctcgctggcgaacgccaatg
ccgccaccgccaccgaactgaccccctcgccccgccctcccccgggggtgtcgacgcggacgtggtcggtcgaggacttcgtcgagct
cttcgagcggaagtacgggcggccgatgacccaagacgaacgcaatgccctcgcgcggggctgcatcggcgtcacgacggtcaacctcga
gcggggcaacatcaacccgccgctcggcatgtcgttcggcacgttcgcgacgtcgcgggacgtgcagaacgcgatcaacgacatcctcgcga
ccaatccctcccggacccaatttgtggcggccgtggcgcagcacccgctcctctcgcggatcgacaacgtcacggactcgctcccgggcggc
ccgacgtcgcagtggacggcggtcatcttctcgaagcggttctactcgaagcaagacccgtcgtggacggacgagcaagcggatcaagcgttc
cgccccgatccggcgacgggccaagtcgacatgacggactaccggtaccgggcgaagccgggctacgtcaacttcgactacggctggctcg
acgagggctcgggcaactggtggcacgcgaaccacgcggagccggggatgaaggtgtatcagtcgacgctccggcactactcgcggccgc
tcctcgacttcgaccggcaagtcttctcggtcacgttcggccgggtccacccg

[0213]

Example 7: Evaluation of protein deamidation activity and transglutaminase activity on protein substrate

(Method)

5 mg of Insulin B chain (insulin B chain, oxidized form, derived from bovine pancreas, Sigma-Aldrich, sequence: Phe-Val-Asn-Gln-His-Leu-Cys(SO_3H)-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys(SO_3H)-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala (SEQ ID NO: 5)) was dissolved in 2 mL of ultrapure water to prepare a substrate solution. Thereafter, 10 μL of a 0.2 mg/mL each purified enzyme solution, 25 μL of a 0.1 M sodium phosphate buffer (pH 6.5), and 0.9 μL of protease inhibitor (ProteoGuard EDTA-Free Protease Inhibitor Cocktail (TaKaRa)) were

added to 22.5 μL of the substrate solution to a total volume of 58.4 μL, so as to prepare a reaction solution. The reaction solution was heated to 40°C in an incubator and was then left at rest overnight. Thereafter, the reaction solution was heated at 100°C for 10 minutes to stop the reaction. The described reaction stop solution was analyzed using HPLC. Deamidation activity and transglutaminase activity were detected by the peak transition from the insulin B chain to the deamidated insulin B chain.

< Protein deamidation activity >

The amount of the deamidated insulin B chain produced was calculated from the peak areas of the insulin B chain and the deamidated insulin B chain that were calculated from the chromatogram, and the protein deamidation activity was then evaluated using the deamidation percentage (amount of deamidated insulin B chain produced ÷ amount of substrate insulin B chain x 100).

(Transglutaminase activity)

The residual amount of insulin B chain and the amount of deamidated insulin B chain produced were calculated from each peak area obtained from the chromatogram, and the cross-linking percentage was evaluated using the formula: (100 – (residual insulin B chain amount / substrate insulin B chain amount x 100) – deamidation percentage).

The ratio between the protein deamidation activity and the transglutaminase activity was calculated from the ratio between the deamidation percentage and the cross-linking percentage calculated by the aforementioned method.

[0214]

(Results)

The results are shown in the following table.

[Table 53]

	Example	Example
	Present enzyme 2 (derived from *C. cryophila*)	Present enzyme 3 (derived from *K. cinnamomea*)
Protein deamidation percentage (%)	44	20
Transglutaminase activity percentage (%)	56	80
Protein deamidation activity / transglutaminase activity	0.78	0.25

[0215]

(Consideration)

In the results shown in the above table, it was demonstrated that the present enzyme 2 and the present enzyme 3 of the present invention have a protein deamidation activity and a transglutaminase activity. Moreover, when the stickiness of "the present enzyme 2" was evaluated following the method of Example 2, an increase in the stickiness could be confirmed. It is assumed that both "the present enzyme 2" (*C. cryophila*) and "the present enzyme 3" (*K. cinnamomea*) would yield effects similar to those of "the present enzyme" (*L. tulufanense*).

[0216]

Example 8: Preparation of enzyme having protein deamidation activity and transglutaminase activity derived from *Longispora fulva* (the present enzyme 4)

A cell mass of *Longispora fulva* was cultured in Trypto-Sawyer Broth Medium (manufactured by Nissui Pharmaceutical Co., Ltd.) at 28°C for 6 days, and the supernatant was then recovered. The obtained supernatant was dialyzed with 20 mM phosphate buffer (pH 6) according to an ordinary method. The dialyzed sample was subjected to ion exchange chromatography using a cation exchange column (SP Sepharose FF, manufactured by GE HealthCare) equilibrated with the same buffer as described above. The active fraction was desalted and concentrated to obtain a crude purified enzyme sample.

[0217]

The amino acid sequences and nucleotide sequences of an enzyme having a protein deamidation activity and a transglutaminase activity, derived from *Longispora fulva*, are shown below.

Amino acid sequence (only mature form sequence) (SEQ ID NO: 14)

APPTSADRSGTPVAGLRVPSSLSAVRGAARFDAVAPEATPLPVGQKTRTWTIARYAAAW
RTANGRAMTDRERDALARGCIGVTTVNIERGNINPPLGLSFGTFEKATAVRDALDAVLA
GAAGRTDYAARVGADPLLSTLDNVTESLPAGDPDQWAAVVFSKRFHSGQDSTWTAAET
DRAFRPDARGQVDMSAYHYRAKPGYVNFDYGWYDPATGSWWHANHAEPGMEIYQST
LKYYSRPLLDFDRQVYSVTFARKAAPAWRSSTN

[0218]

Amino acid sequence (full length) * The underlined part indicates the mature body sequence. (SEQ ID NO: 15)

MRNRLLRITMAAVLGVGATVGLAATTGYA<u>APPTSADRSGTPVAGLRVPSSLSAVRGAAR
FDAVAPEATPLPVGQKTRTWTIARYAAAWRTANGRAMTDRERDALARGCIGVTTVNIE
RGNINPPLGLSFGTFEKATAVRDALDAVLAGAAGRTDYAARVGADPLLSTLDNVTESLP
AGDPDQWAAVVFSKRFHSGQDSTWTAAETDRAFRPDARGQVDMSAYHYRAKPGYVNF
DYGWYDPATGSWWHANHAEPGMEIYQSTLKYYSRPLLDFDRQVYSVTFARKAAPAWR
SSTN</u>

[0219]

Nucleotide sequence (mature body) (SEQ ID NO: 16)

gccccgccgacgtccgccgaccgctcggggacccccgtggcggggctgcgggtcccgtcgtccctctccgccgtgcgcggcgccgcccgg
tttgacgccgtggcgccggaagccaccccgctcccggtcgggcaaaagacccgcacgtggacgattgcccgctacgccgcggcctggcgg
accgcgaacggccgcgccatgaccgatcgggagcgcgatgccctcgcccggggctgcatcggcgtcacgacggtcaacatcgagcgggg
caacatcaacccgccgctcggcctctccttcggcacgtttgaaaaagcgaccgcggtccgcgacgcgctggacgccgtcctcgccggggccg
cgggccgcaccgactacgccgcgcgcgtcggcgcggatccgctcctctccacgctcgataatgtcacggaatcgctcccggcgggggaccc
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cgatgcgcgcggccaagtcgacatgtcggcgtaccactaccgggcgaagccgggctacgtcaacttcgactacggctggtacgacccggcg
acgggctcgtggtggcacgcgaaccacgcggagccgggcatggagatctatcagtcgacgctcaagtactactcgcggccgctcctcgacttc
gaccggcaagtctactcggtcacgttcgcgcggaaggcggcgccggcgtggcggtcgtcgacgaac

[0220]

Nucleotide sequence (full length) (SEQ ID NO: 17)

atgcggaaccgcctgctccggatcacgatggccgcggtcctgggggtgggcgccaccgtggggctcgccgcgaccacgggctatgcggcc
ccgccgacgtccgccgaccgctcggggacccccgtggcggggctgcgggtcccgtcgtccctctccgccgtgcgcggcgccgcccggtttg
acgccgtggcgccggaagccaccccgctcccggtcgggcaaaagacccgcacgtggacgattgcccgctacgccgcggcctggcggacc
gcgaacggccgcgccatgaccgatcgggagcgcgatgccctcgcccggggctgcatcggcgtcacgacggtcaacatcgagcggggcaa
catcaacccgccgctcggcctctccttcggcacgtttgaaaaagcgaccgcggtccgcgacgcgctggacgccgtcctcgccggggccgcgg
gccgcaccgactacgccgcgcgcgtcggcgcggatccgctcctctccacgctcgataatgtcacggaatcgctcccggcgggggacccggat
cagtgggcggcggtcgtcttctcgaagcgctttcattccgggcaagattccacctggaccgccgcggagaccgaccgggcgtttcggcccgat
gcgcgcggccaagtcgacatgtcggcgtaccactaccgggcgaagccgggctacgtcaacttcgactacggctggtacgacccggcgacgg
gctcgtggtggcacgcgaaccacgcggagccgggcatggagatctatcagtcgacgctcaagtactactcgcggccgctcctcgacttcgacc
ggcaagtctactcggtcacgttcgcgcggaaggcggcgccggcgtggcggtcgtcgacgaac

[0221]

Example 9: Evaluation of protein deamidation activity and transglutaminase activity on protein substrate

(Method)

5 mg of Insulin B chain (insulin B chain, oxidized form, derived from bovine pancreas, Sigma-Aldrich, sequence: Phe-Val-Asn-Gln-His-Leu-Cys(SO_3H)-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys(SO_3H)-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala (SEQ ID NO: 5)) was dissolved in 2 mL of ultrapure water to prepare a substrate solution. Thereafter, 40 μL of a 1 mg/mL purified enzyme solution and a 0.1 M potassium dihydrogen phosphate/disodium hydrogen phosphate buffer (pH 6.5) were added to 90 μL of the substrate solution to a total volume of 180 μL, so as to prepare a reaction solution. The reaction solution was heated to 40°C in an incubator and was then left at rest for 5 hours. Thereafter, the reaction solution was heated at 100°C for 10 minutes to stop the reaction. The reaction stop solution was analyzed using HPLC. Deamidation activity and transglutaminase activity were detected by the peak transition from the insulin B chain to the deamidated insulin B chain.

[0222]

(Results)

As a result of the analysis using HPLC, a peak based on the deamidation reaction was confirmed in the case of using the present enzyme 4. In addition, it was assumed that the present enzyme 4 would have a transglutaminase activity.

Industrial Applicability

[0223]

The enzyme agent of the present invention contains, as an active ingredient, an enzyme having a protein deamidation activity and a transglutaminase activity. Therefore, the present enzyme agent is suitable to be used in the fields of food products and medical applications, and is industrially highly useful.

[0224]

The present invention is not limited in any way to the above-described embodiments and examples of the present invention. Various modifications are also included in the present invention, in such a range that those skilled in the art can readily conceive of them, and that the modifications are not deviated from the scope of the claims. The contents of the study papers, laid-open patent publications, patent publications, etc., which are explicitly mentioned in the present description, are cited herein by reference in their entirety.

CLAIMS

[Claim 1]

An enzyme agent for protein deamidation and protein cross-linking, the enzyme agent containing, as an active ingredient, an enzyme that consists of an amino acid sequence having a sequence identity of 90% or more to the amino acid sequence as set forth in SEQ ID NO: 1 and has a protein deamidation activity and a transglutaminase activity.

[Claim 2]

The enzyme agent for protein deamidation and protein cross-linking according to claim 1, in which the enzyme is derived from Longimycelium sp.

[Claim 3]

A method for deamidating and cross-linking a protein or a peptide, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 1 or 2 to act on the protein or the peptide.

[Claim 4]

A method for deamidating and cross-linking protein-containing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 1 or 2 to act on the protein-containing food product and beverage.

[Claim 5]

A method for producing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 1 or 2 to act on protein-containing food product and beverage.

[Claim 6]

Food product and beverage, treated with the enzyme agent for protein deamidation and protein cross-linking according to claim 1 or 2.

[Claim 7]

A method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 90% or more to the amino acid sequence as set forth in SEQ ID NO: 1, including

culturing bacteria of Longimycelium sp.

[Claim 8]

An enzyme agent for protein deamidation and protein cross-linking, the enzyme agent containing, as an active ingredient, an enzyme that consists of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 6 and has a protein deamidation activity and a transglutaminase activity.

[Claim 9]

The enzyme agent for protein deamidation and protein cross-linking according to claim 8, in which the enzyme is derived from Crossiella sp.

[Claim 10]

A method for deamidating and cross-linking a protein or a peptide, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 8 or 9 to act on the protein or the peptide.

[Claim 11]

A method for deamidating and cross-linking protein-containing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 8 or 9 to act on the protein-containing food product and beverage.

[Claim 12]

A method for producing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 8 or 9 to act on protein-containing food product and beverage.

[Claim 13]

Food product and beverage, treated with the enzyme agent for protein deamidation and protein cross-linking according to claim 8 or 9.

[Claim 14]

A method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 6, including

culturing bacteria of Crossiella sp.

[Claim 15]

An enzyme agent for protein deamidation and protein cross-linking, the enzyme agent containing, as an active ingredient, an enzyme that consists of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 10 and has a protein deamidation activity and a transglutaminase activity.

[Claim 16]

The enzyme agent for protein deamidation and protein cross-linking according to claim 15, in which the enzyme is derived from Krasilnikovia sp.

[Claim 17]

A method for deamidating and cross-linking a protein or a peptide, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 15 or 16 to act on the protein or the peptide.

[Claim 18]

A method for deamidating and cross-linking protein-containing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 15 or 16 to act on the protein-containing food product and beverage.

[Claim 19]

A method for producing food product and beverage, including

allowing the enzyme agent for protein deamidation and protein cross-linking according to claim 15 or 16 to act on protein-containing food product and beverage.

[Claim 20]

Food product and beverage, treated with the enzyme agent for protein deamidation and protein cross-linking according to claim 15 or 16.

[Claim 21]

A method for producing a protein deamidation and protein cross-linking enzyme consisting of an amino acid sequence having a sequence identity of 65% or more to the amino acid sequence as set forth in SEQ ID NO: 10, including

culturing bacteria of Krasilnikovia sp.